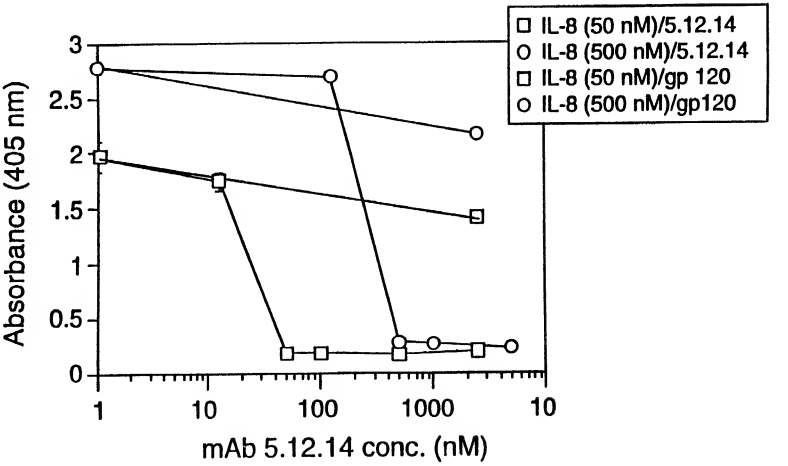
FIG. 1


IL-8 (50 nM)/5.12.14
IL-8 (500 nM)/5.12.14
IL-8 (50 nM)/gp 120
IL-8 (500 nM)/gp120
Absorbance (405 nm)
3
2.5
2
1.5
1
0.5
0
1
10
100
1000
10
mAb 5.12.14 conc. (nM)

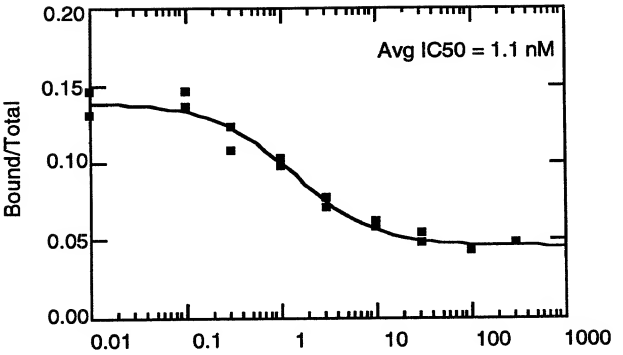

Avg IC50 = 1.1 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
unlabeled IL8 (nM)

FIG. 2

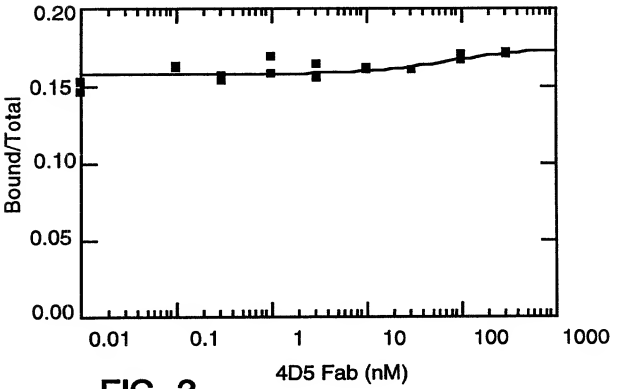

Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
4D5 Fab (nM)

FIG. 3

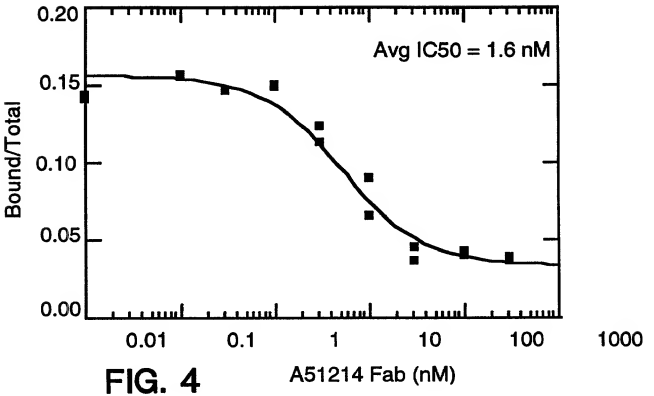

Avg IC50 = 1.6 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
A51214 Fab (nM)

FIG. 4

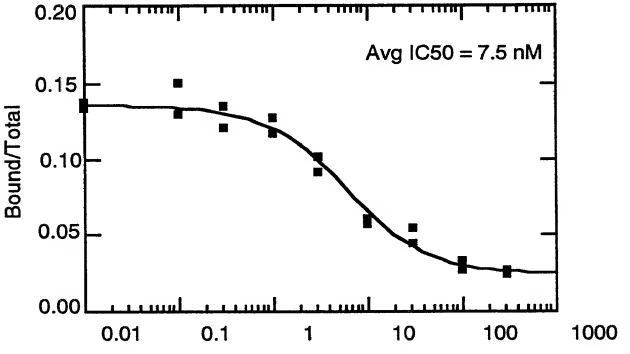

Avg IC50 = 7.5 nM
Bound/Total
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
6G425 Fab (nM)

FIG. 5

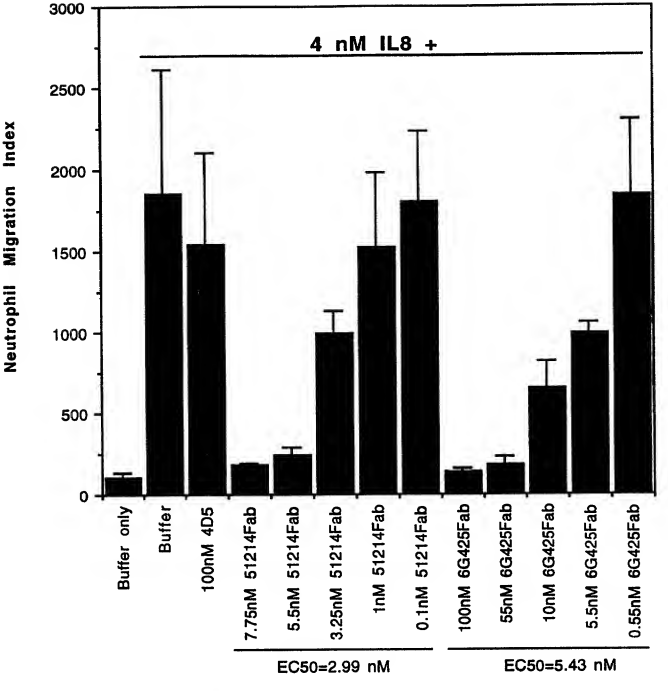

Neutrophil Migration Index
3000
2500
2000
1500
1000
500
0
4 nM IL8 +
Buffer only
Buffer
100nM 4D5
7.75nM 51214Fab
5.5nM 51214Fab
3.25nM 51214Fab
1nM 51214Fab
0.1nM 51214Fab
100nM 6G425Fab
55nM 6G425Fab
10nM 6G425Fab
5.5nM 6G425Fab
0.55nM 6G425Fab
EC50=2.99 nM
EC50=5.43 nM

FIG. 6

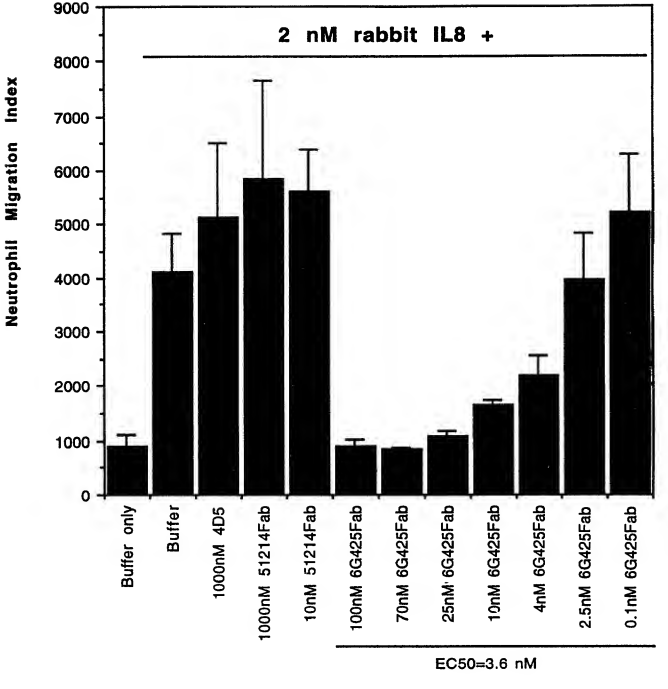

Neutrophil Migration Index
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2 nM rabbit IL8 +
Buffer only
Buffer
1000nM 4D5
1000nM 51214Fab
10nM 51214Fab
100nM 6G425Fab
70nM 6G425Fab
25nM 6G425Fab
10nM 6G425Fab
4nM 6G425Fab
2.5nM 6G425Fab
0.1nM 6G425Fab
EC50=3.6 nM

FIG. 7

FIG. 8

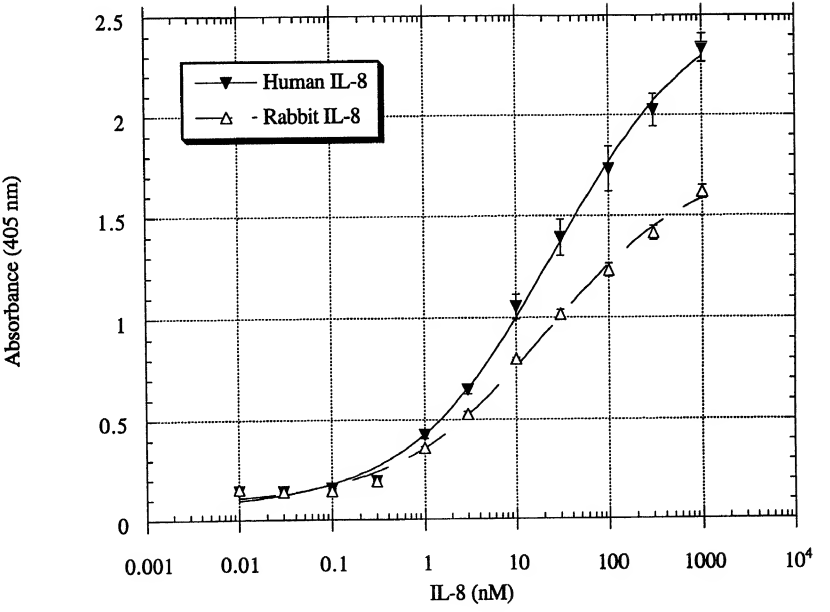
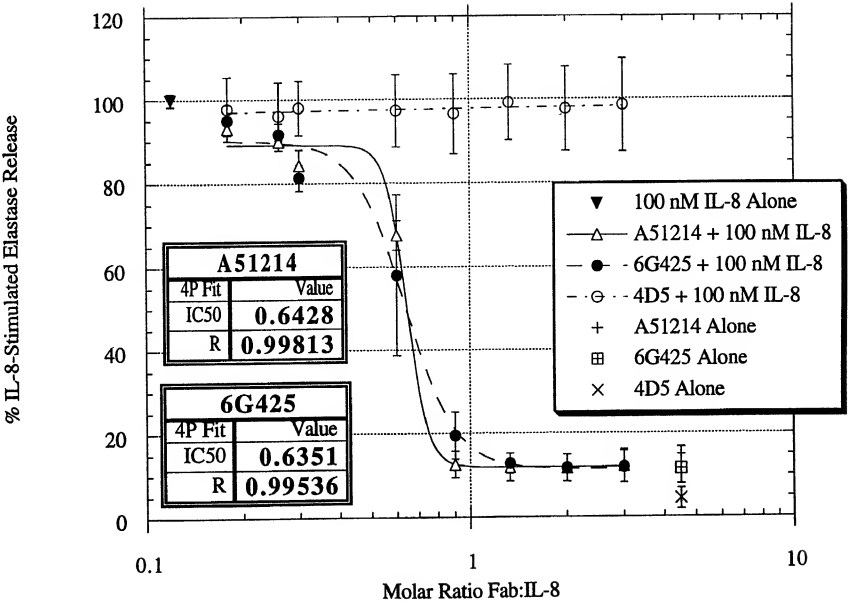
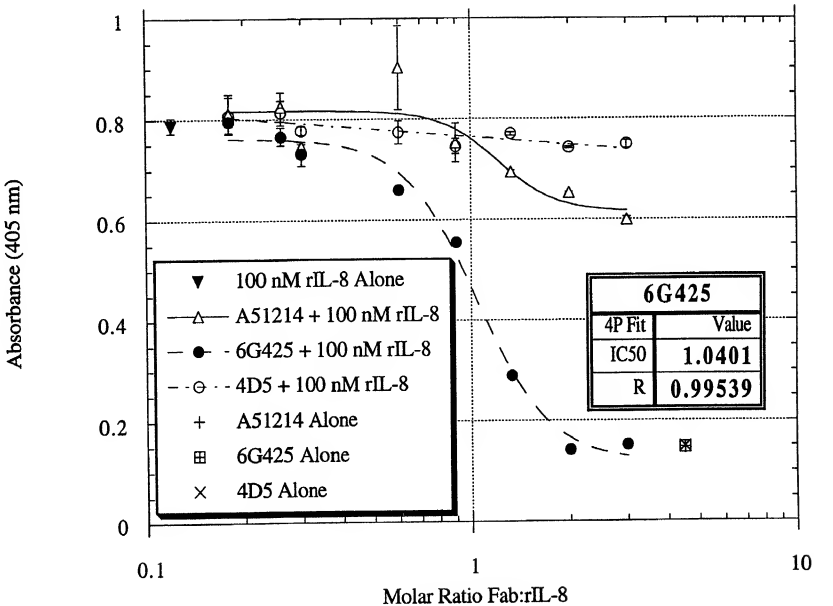

Human IL-8
- Rabbit IL-8
Absorbance (405 nm)
2.5
2
1.5
1
0.5
0
0.001
0.01
0.1
1
10
100
1000
10^4
IL-8 (nM)

FIG. 9


120
100
80
60
40
20
0
0.1
1
10
% IL-8-Stimulated Elastase Release
Molar Ratio Fab:IL-8
A51214
4P Fit | Value
IC50 | 0.6428
R | 0.99813
6G425
4P Fit | Value
IC50 | 0.6351
R | 0.99536
100 nM IL-8 Alone
A51214 + 100 nM IL-8
6G425 + 100 nM IL-8
4D5 + 100 nM IL-8
A51214 Alone
6G425 Alone
4D5 Alone

Scanned

FIG. 10



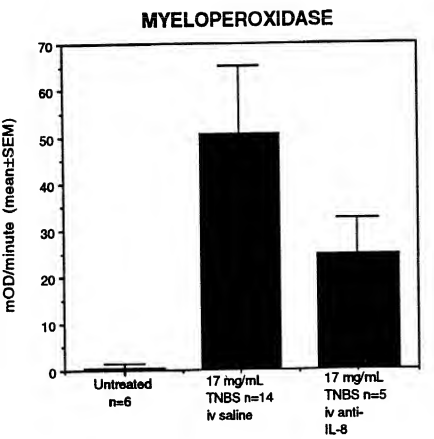


FIG. 11A

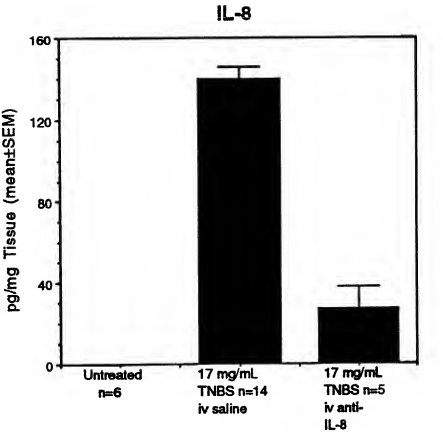


FIG. 11B

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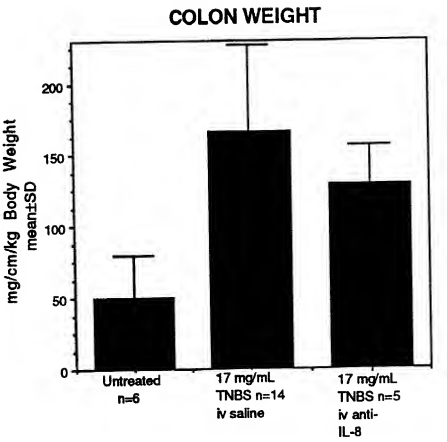


FIG. 11C

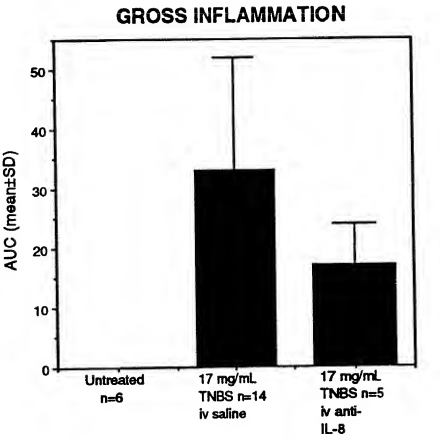


FIG. 11D

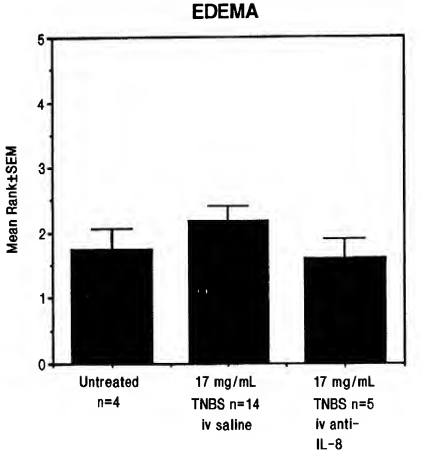


FIG. 11E

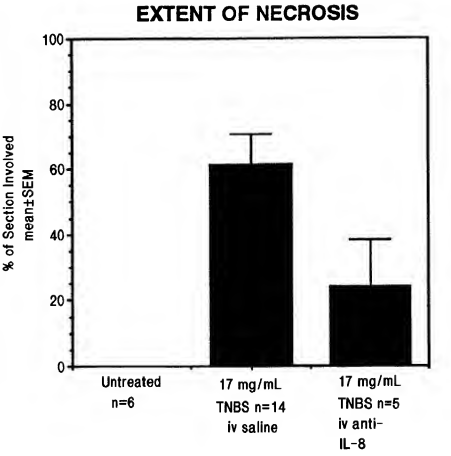


FIG. 11F

SUBSTITUTE SHEET (RULE 26)

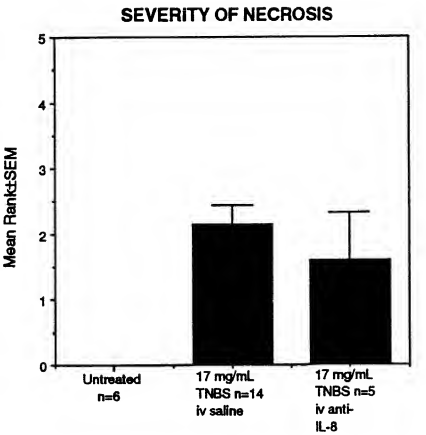


FIG. 11G

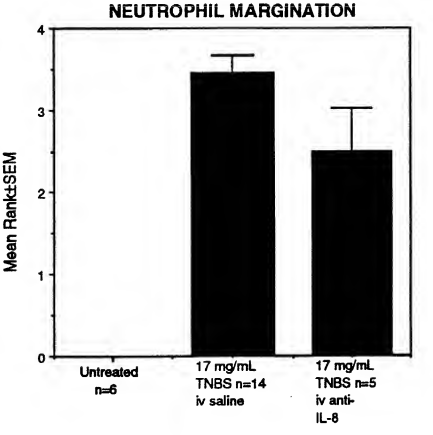


FIG. 11H

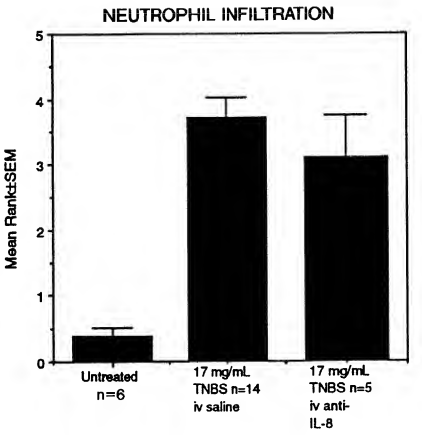


FIG. 11I

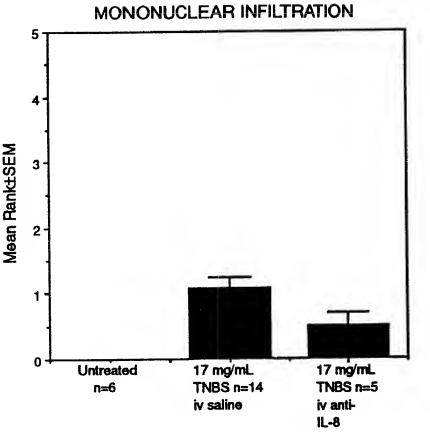
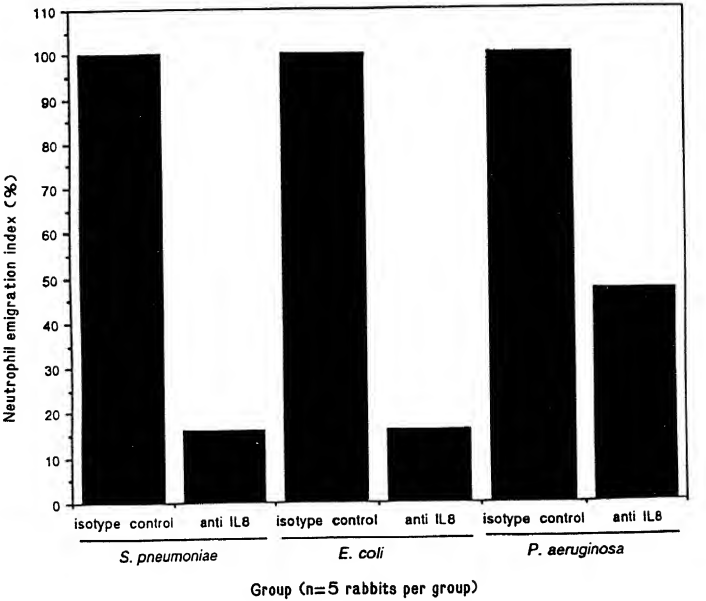


FIG. 11J

Under 37 CFR 1.84

FIG. 12



Light Chain Primers:

FIG. 13

MKLC-1, 22mer

5' CAGTCCAACTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

FIG. 14

Light chain forward primer

SL001A-2 35 mer

5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC 3'
T T T
A

Light chain reverse primer

SL001B 37 mer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3'

Heavy chain forward primer

FIG. 15

SL002B 39 mer

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3'
                        T           C
                        G
                        A
```

Heavy chain reverse primer

SL002B 39-MER

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC  3'
                        T
                        A
                        G
```

```
  1 GACATTGTCA TGACACAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA CAGGGTCAGC
    CTGTAACAGT ACTGTGTCAG AGTTTTTAAG TACAGGTGTA GTCATCCTCT GTCCCAGTCG
  1 D  I  V  M   T  Q  S   Q  K  F   M  S  T  S   V  G  D   R  V  S

 61 GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG CCTGGTATCA ACAGAAACCA
    CAGTGGACGT TCCGGTCAGT CTTACACCCA TGATTACATC GGACCATAGT TGTCTTTGGT
 21 V  T  C  K   A  S  Q   N  V  G   T  N  V  A   W  Y  Q   Q  K  P
               *   *  *  *   *  *  *   *  *  *  *
                                CDR #1

121 GGGCAATCTC CTAAAGCACT GATTTACTCG TCATCCTACC GGTACAGTGG AGTCCCTGAT
    CCCGTTAGAG GATTTCGTGA CTAAATGAGC AGTAGGATGG CCATGTCACC TCAGGGACTA
 41 G  Q  S  P   K  A  L   I  Y  S   S  S  Y  R   Y  S  G   V  P  D
                              *   *  *  *  *   *  *
                                CDR #2

181 CGCTTCACAG GCAGTGGATC TGGGACAGAT TTCACTCTCA CCATCAGCCA TGTGCAGTCT
    GCGAAGTGTC CGTCACCTAG ACCCTGTCTA AAGTGAGAGT GGTAGTCGGT ACACGTCAGA
 61 R  F  T  G   S  G  S   G  T  D   F  T  L  T   I  S  H   V  Q  S

241 GAAGACTTGG CAGACTATTT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT
    CTTCTGAACC GTCTGATAAA GACAGTCGTT ATATTGTAGA TAGGAGAGTG CAAGCCAGGA
 81 E  D  L  A   D  Y  F   C  Q  Q   Y  N  I  Y   P  L  T   F  G  P
                            *  *   *  *  *  *   *  *  *
                                CDR #3

301 GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC CATCTTCCCA
    CCCTGGTTCG ACCTCAACTT TGCCCGACTA CGACGTGGTG GTTGACATAG GTAGAAGGGT
101 G  T  K  L   E  L  K   R  A  D   A  A  P  P   T  V  S   I  F  P

      BstBI
361 CCATTCGAA
    GGTAAGCTT
121 P  F  E
```

FIG. 16

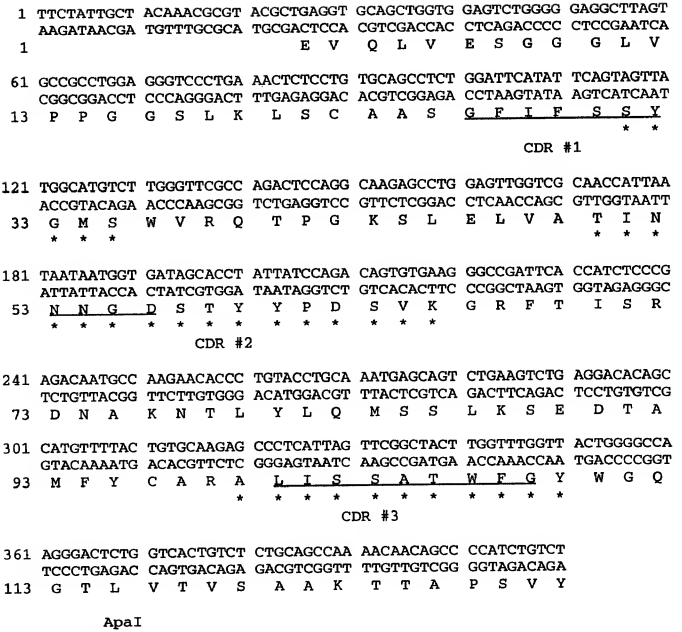

1 TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG GAGGCTTAGT
AAGATAACGA TGTTTGCGCA TGCGACTCCA CGTCGACCAC CTCAGACCCC CTCCGAATCA
1 E V Q L V E S G G G L V
61 GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT GGATTCATAT TCAGTAGTTA
CGGCGGACCT CCCAGGGACT TTGAGAGGAC ACGTCGGAGA CCTAAGTATA AGTCATCAAT
13 P P G G S L K L S C A A S G F I F S S Y
CDR #1
121 TGGCATGTCT TGGGTTCGCC AGACTCCAGG CAAGAGCCTG GAGTTGGTCG CAACCATTAA
ACCGTACAGA ACCCAAGCGG TCTGAGGTCC GTTCTCGGAC CTCAACCAGC GTTGGTAATT
33 G M S W V R Q T P G K S L E L V A T I N
181 TAATAATGGT GATAGCACCT ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG
ATTATTACCA CTATCGTGGA TAATAGGTCT GTCACACTTC CCGGCTAAGT GGTAGAGGGC
53 N N G D S T Y Y P D S V K G R F T I S R
CDR #2
241 AGACAATGCC AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC
TCTGTTACGG TTCTTGTGGG ACATGGACGT TTACTCGTCA GACTTCAGAC TCCTGTGTCG
73 D N A K N T L Y L Q M S S L K S E D T A
301 CATGTTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT ACTGGGGCCA
GTACAAAATG ACACGTTCTC GGGAGTAATC AAGCCGATGA ACCAAACCAA TGACCCCGGT
93 M F Y C A R A L I S S A T W F G Y W G Q
CDR #3
361 AGGGACTCTG GTCACTGTCT CTGCAGCCAA AACAACAGCC CCATCTGTCT
TCCCTGAGAC CAGTGACAGA GACGTCGGTT TTGTTGTCGG GGTAGACAGA
113 G T L V T V S A A K T T A P S V Y
ApaI
411 ATCCGGG
TAGGCCC
130 P

FIG. 17

FIG. 18

VL.front 31-MER

5' ACAAACGCGTACGCTGATATCGTCATGACAG 3'

VL.rear 31-MER

5' GCAGCATCAGCTCTTCGAAGCTCCAGCTTGG 3'

VH.front.SPE 21-MER

5' CCACTAGTACGCAAGTTCACG 3'

VH.rear 33-MER

5' GATGGGCCCTTGGTGGAGGCTGCAGAGACAGTG 3'

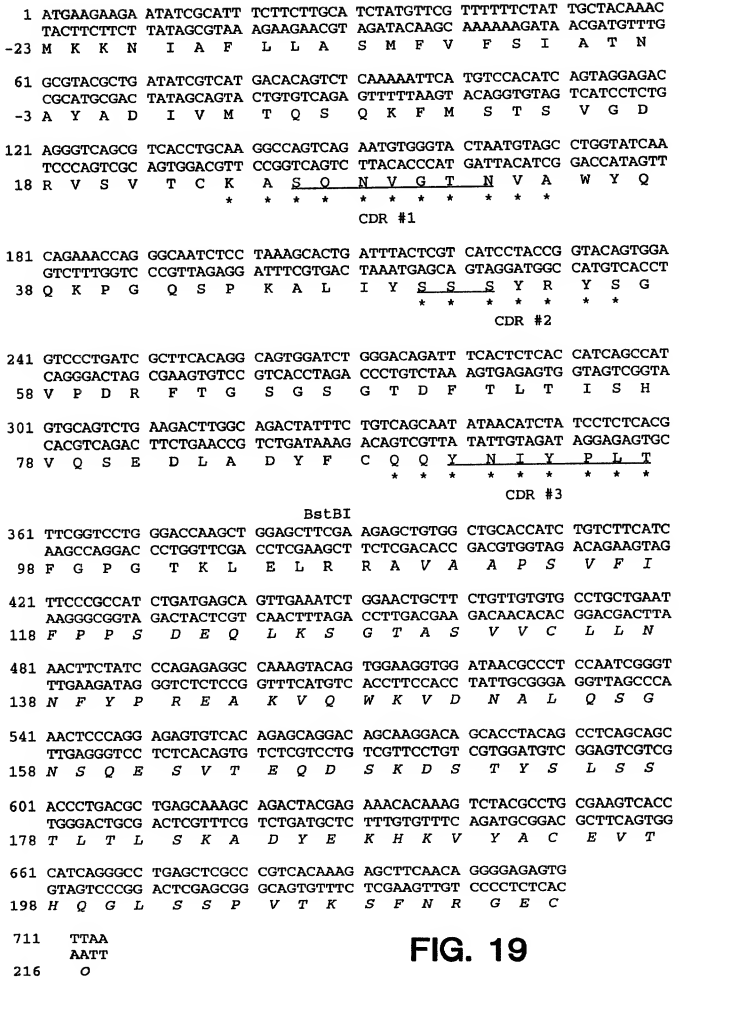


FIG. 19

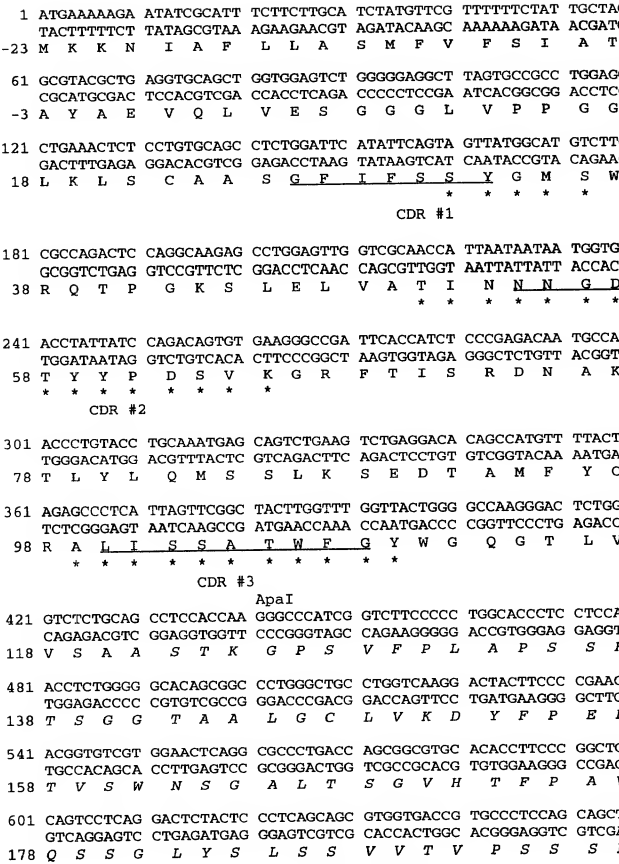

1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M K K N I A F L L A S M F V F S I A T N
61 GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT TAGTGCCGCC TGGAGGGTCC
CGCATGCGAC TCCACGTCGA CCACCTCAGA CCCCCTCCGA ATCACGGCGG ACCTCCCAGG
-3 A Y A E V Q L V E S G G G L V P P G G S
121 CTGAAACTCT CCTGTGCAGC CTCTGGATTC ATATTCAGTA GTTATGGCAT GTCTTGGGTT
GACTTTGAGA GGACACGTCG GAGACCTAAG TATAAGTCAT CAATACCGTA CAGAACCCAA
18 L K L S C A A S G F I F S S Y G M S W V
* * * *
CDR #1
181 CGCCAGACTC CAGGCAAGAG CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC
GCGGTCTGAG GTCCGTTCTC GGACCTCAAC CAGCGTTGGT AATTATTATT ACCACTATCG
38 R Q T P G K S L E L V A T I N N N G D S
* * * * * * *
241 ACCTATTATC CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC
TGGATAATAG GTCTGTCACA CTTCCCGGCT AAGTGGTAGA GGGCTCTGTT ACGGTTCTTG
58 T Y Y P D S V K G R F T I S R D N A K N
* * * * * * * *
CDR #2
301 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT TTACTGTGCA
TGGGACATGG ACGTTTACTC GTCAGACTTC AGACTCCTGT GTCGGTACAA AATGACACGT
78 T L Y L Q M S S L K S E D T A M F Y C A
361 AGAGCCCTCA TTAGTTCGGC TACTTGGTTT GGTTACTGGG GCCAAGGGAC TCTGGTCACT
TCTCGGGAGT AATCAAGCCG ATGAACCAAA CCAATGACCC CGGTTCCCTG AGACCAGTGA
98 R A L I S S A T W F G Y W G Q G T L V T
* * * * * * * * * *
CDR #3
ApaI
421 GTCTCTGCAG CCTCCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCTC CTCCAAGAGC
CAGAGACGTC GGAGGTGGTT CCCGGGTAGC CAGAAGGGGG ACCGTGGGAG GAGGTTCTCG
118 V S A A S T K G P S V F P L A P S S K S
481 ACCTCTGGGG GCACAGCGGC CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG
TGGAGACCCC CGTGTCGCCG GGACCCGACG GACCAGTTCC TGATGAAGGG GCTTGGCCAC
138 T S G G T A A L G C L V K D Y F P E P V
541 ACGGTGTCGT GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA
TGCCACAGCA CCTTGAGTCC GCGGGACTGG TCGCCGCACG TGTGGAAGGG CCGACAGGAT
158 T V S W N S G A L T S G V H T F P A V L
601 CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG CAGCTTGGGC
GTCAGGAGTC CTGAGATGAG GGAGTCGTCG CACCACTGGC ACGGGAGGTC GTCGAACCCG
178 Q S S G L Y S L S S V V T V P S S S L G

FIG. 20A

```
661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA
    TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT TGTGGTTCCA CCTGTTCTTT
198 T  Q  T  Y   I  C  N   V  N  H   K  P  S  N   T  K  V   D  K  K

721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGA
    CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT
218 V  E  P  K   S  C  D   K  T  H   T  O
```

FIG. 20B

Light Chain Primers:

MKLC-1, 22mer

5' CAGTCCAACTGTTCAGGACGCC 3'

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3'

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3'

Heavy Chain Primers:

IGG2AC-1, 24mer

5' GCATCCTAGAGTCACCGAGGAGCC 3'

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3'

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3'

FIG. 21

Light chain forward primer

6G4.light.Nsi 36-MER

```
5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC  3'
                  T   T           T       T
                                  A       A
```

Light chain reverse primer

6G4.light.Mun 35-MER

```
5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3'
```

FIG. 22

Heavy chain forward primer

6G4.heavy.Mlu 32-MER

```
5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG  3'
                     T       C
```

Heavy chain reverse primer

SL002B 39-MER

```
5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC  3'
                T
                A
                G
```

FIG. 23

```
 70 G ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT
    C TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
  1 D   I   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D

121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
    GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
 18 Q   A   S   I   S   C   R   S   S   Q   S   L   V   H   G   I   G   N   T   Y
                        *   *   *   *   *   *   *   *   *   *   *   *   *   *
                                                CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
    AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
 38 L   H   W   Y   L   Q   K   P   G   Q   S   P   K   L   L   I   Y   K   V   S
    *   *                                                           *   *   *
                                                                    CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA
    TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N   R   F   S   G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T
    *   *   *   *
301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
    GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
 78 L   R   I   S   R   V   E   A   E   D   L   G   L   Y   F   C   S   Q   S   T
                                                            *   *   *   *
                                                            CDR #3

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGATGCTGCA
    GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACTACGACGT
 98 H   V   P   L   T   F   G   A   G   T   K   L   E   L   K   R   A   D   A   A
    *   *   *   *   *
                                                MunI
421 CCAACTGTAT CCATCTTCCC ACCATCCAGT GAGCAATTGA
    GGTTGACATA GGTAGAAGGG TGGTAGGTCA CTCGTTAACT
118 P   T   V   S   I   F   P   P   S   S   E   Q   L   K
```

FIG. 24

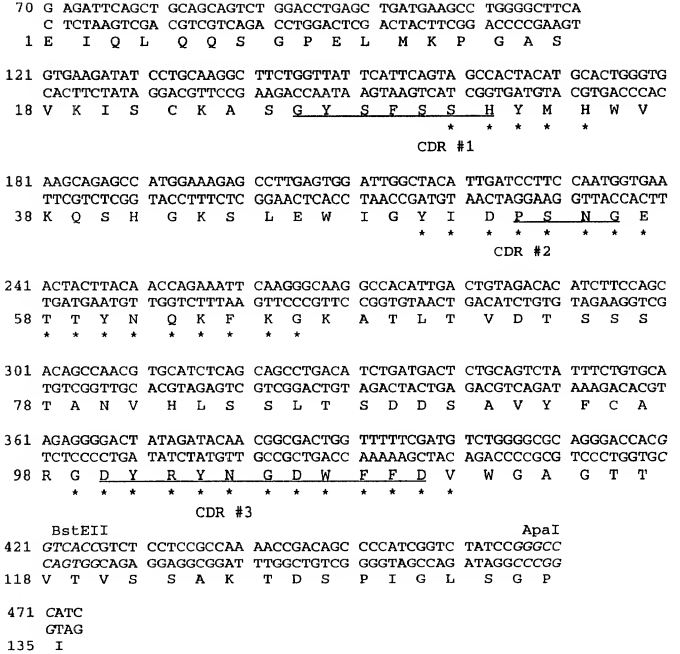

70 G AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
C TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
1 E I Q L Q Q S G P E L M K P G A S
121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTCAGTA GCCACTACAT GCACTGGGTG
CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
18 V K I S C K A S G Y S F S S H Y M H W V
CDR #1
181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
TTCGTCTCGG TACCTTTCTC GGAACTCACC TAACCGATGT AACTAGGAAG GTTACCACTT
38 K Q S H G K S L E W I G Y I D P S N G E
CDR #2
241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAACT GACATCTGTG TAGAAGGTCG
58 T T Y N Q K F K G K A T L T V D T S S S
301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
78 T A N V H L S S L T S D D S A V Y F C A
361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
TCTCCCCTGA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
98 R G D Y R Y N G D W F F D V W G A G T T
CDR #3
BstEII ApaI
421 GTCACCGTCT CCTCCGCCAA AACCGACAGC CCCATCGGTC TATCCGGGCC
CAGTGGCAGA GGAGGCGGAT TTGGCTGTCG GGGTAGCCAG ATAGGCCCGG
118 V T V S S A K T D S P I G L S G P
471 CATC
GTAG
135 I

FIG. 25

5' CTTGGTGGAGGCGGAGGAGACG 3'

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATTCATCTTCC 3'

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3'

SYN.Apa 22 MER

5' CTTGGTGGAGGCGGAGGAGACG 3'

FIG. 26

```
  1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAT
    TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTA
-23 M  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT
    CGTATGCGAC TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
 -3 A  Y  A  D   I  V  M   T  Q  T   P  L  S  L   P  V  S   L  G  D

121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
    GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA
 18 Q  A  S  I   S  C  R   S  S  Q   S  L  V  H   G  I  G   N  T  Y
                  *  *  *   *  *  *   *  *  *  *   *  *  *   *  *  *
                                        CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
    AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG
 38 L  H  W  Y   L  Q  K   P  G  Q   S  P  K  L   L  I  Y   K  V  S
    *  *                                                     *  *  *
                                                            CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA
    TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N  R  F  S   G  V  P   D  R  F   S  G  S  G   S  G  T   D  F  T
    *  *  *  *
                                                            CDR #3

301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA
    GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT
 78 L  R  I  S   R  V  E   A  E  D   L  G  L  Y   F  C  S   Q  S  T
                                                    *  *  *   *  *  *

361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGTTGCTGCA
    GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACAACGACGT
 98 H  V  P  L   T  F  G   A  G  T   K  L  E  L   K  R  A   V  A  A
    *  *  *  *   *

421 CCAACTGTAT TCATCTTCCC ACCATCCAGT GAGCAATTGA AATCTGGAAC TGCCTCTGTT
    GGTTGACATA AGTAGAAGGG TGGTAGGTCA CTCGTTAACT TTAGACCTTG ACGGAGACAA
118 P  T  V  F   I  F  P   P  S  S   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y
```

FIG. 27A

SNEDTT:2E292469

```
661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G


721 GAGTGTTAA
    CTCACAATT
218 E  C  O
```

FIG. 27B

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N    I  A  F    L  L  A    S  M  F  V    F  S  I    A  T  N

 61 GCGTACGCTG AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
    CGCATGCGAC TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
 -3 A  Y  A  E    I  Q  L    Q  Q  S    G  P  E  L    M  K  P    G  A  S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTCAGTA GCCACTACAT GCACTGGGTG
    CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V  K  I  S    C  K  A    S  G  Y  S  F  S  S    H  Y  M    H  W  V
                                                  *  *  *  *    *
                                                CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
    TTCGTCTCGG TACCTTTCTC GGAACTCACC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K  Q  S  H    G  K  S    L  E  W    I  G  Y  I    D  P  S  N  G  E
                                        *  *    *  *  *    *  *  *
                                                   CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
    TGATGAATGT TGGTCTTTAA GTTCCCGTTC CGGTGTAACT GACATCTGTG TAGAAGGTCG
 58 T  T  Y  N    Q  K  F    K  G  K    A  T  L  T    V  D  T    S  S  S
    *  *  *  *    *  *  *    *  *

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
    TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T  A  N  V    H  L  S    S  L  T    S  D  D  S    A  V  Y    F  C  A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
    TCTCCCCTGA TATCTATGTT GCCGCTGACC AAAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R  G  D  Y    R  Y  N    G  D  W    F  F  D  V    W  G  A    G  T  T
       *  *  *    *  *  *    *  *  *    *  *  *  *
                          CDR #3

421 GTCACCGTCT CCTCCGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
    CAGTGGCAGA GGAGGCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG
118 V  T  V  S    S  A  S    T  K  G    P  S  V  F    P  L  A    P  S  S

481 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA
    TTCTCGTGGA GACCCCCGTG TCGCCGGGAC CCGACGGACC AGTTCCTGAT GAAGGGGCTT
138 K  S  T  S    G  G  T    A  A  L    G  C  L  V    K  D  Y    F  P  E

541 CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT
    GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA
158 P  V  T  V    S  W  N    S  G  A    L  T  S  G    V  H  T    F  P  A

601 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC
    CAGGATGTCA GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGGCACGG GAGGTCGTCG
178 V  L  Q  S    S  G  L    Y  S  L    S  S  V  V    T  V  P    S  S  S
```

FIG. 28A

```
661 TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC
    AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG GTTCCACCTG
198 L  G  T  Q   T  Y  I   C  N  V   N  H  K  P   S  N  T   K  V  D

721 AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GA
    TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CT
218 K  K  V  E   P  K  S   C  D  K   T  H  T  O
```

FIG. 28B

Variable Light Chain Domain

```
                10        20        abcde 30        40
6G425    DIVMTQTPLSLPVSLGDQASISCRSSQSLVHGIGNTYLHWYLQKPGQSPKLLIY
           #   # #  ## #  ### #                     #     ##
F(ab)-1  DIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTYLHWYQQKPGKAPKLLIY
                                 # ##########
humκI    DIQMTQSPSSLSASVGDRVTITCRASKTI-----SKYLAWYQQKPGKAPKLLIY
                                 ============
                                 +++++++++++++++
                                      L1

                50        60        70        80        90        100
6G425    YKVSNRFSGVPDRFSDSGSGTDFTLRISRVEAEDLGLYFCSQSTHVPLTFGAGTKLELKR
                   #   #          #  ##### ### #              #   # #
F(ab)-1  YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPLTFGQGTKVEIKR
          ## ###                                  # ####
humκI    YSGSTLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEYPLTFGQGTKVEIKR
          ===                                     ======
          +++++++                                 +++++++++
              L2                                      L3
```

Variable Heavy Chain Domain

```
                10        20        30        40
6G425    EIQLQQSGPELMKPGASVKISCKASGYSFSSHYMHWVKQSHGKSLEWI
          #  ##  ## ##  # ###  #                 # ##  #   #
F(ab)-1  EVQLVESGGGLVQPGGSLRLSCAASGYSFSSHYMHWVRQAPGKGLEWV
                                 #  ## # #
humIII   EVQLVESGGGLVQPGGSLRLSCAASGFSFTGHWMNWVRQAPGKGLEWV
                                 =======
                                    +++++
                                     H1

                50  a                 70        80  abc       90        100       110
6G425    GYIDPSNGETTYNQKFKGKATLTVDTSSSTANVHLSSLTSDDSAVYFCAARGDYRYNGDWFFDVWGAGT
                              ## ### # ## ######  ### #    #                    #
F(ab)-1  GYIDPSNGETTYNQKFKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAARGDYRYNGDWFFDVWGQGT
          # #  ##  # ####                                                # # # ###  #
humIII   GMIHPSDSETRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAARGIYFY-GTTYFDYWGQGT
           ====                                                          ===========
          +++++++++++++++++                                             +++++++++++++
               H2                                                            H3
```

FIG. 29

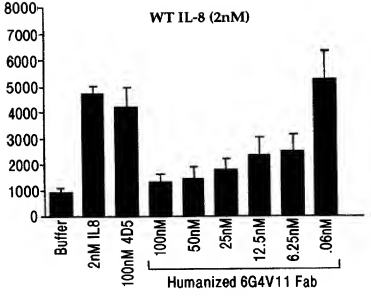


FIG. 30A

IC50~12nM

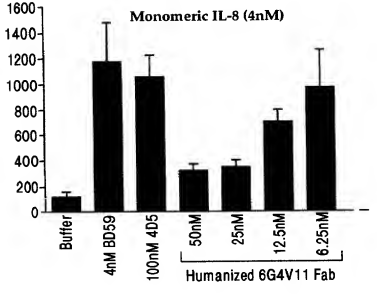


FIG. 30B

IC50~15nM

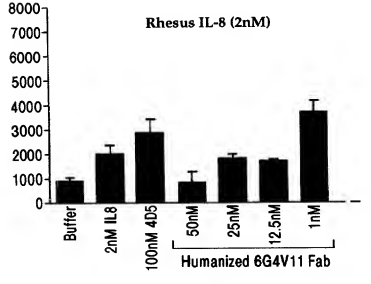


FIG. 30C

IC50~22nM

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLV**Q**SGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFT**L**SRDNSKNT**A**YLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

Amino Acid Sequence of the peptide linker and M13 Phage Coat (gene-III)

SGGGSGSGDFDYEKMANANKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVS
GLANGNGATGDFAGSSNSQMAQVGDGDNSPLMNNFRQYLPSLPQSVECRPFVFSAGKPY
EFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES

FIG. 31A

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  K  N   I  A  F   L  L  A   S  M  F  V   F  S  I   A  T  N

 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A  Y  A  D   I  Q  M   T  Q  S   P  S  S  L   S  A  S   V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TAACACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
 18 R  V  T  I   T  C  R   S  S  Q   S  L  V  H   G  I  G   N  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L  H  W  Y   Q  Q  K   P  G  K   A  P  K  L   L  I  Y   K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N  R  F  S   G  V  P   S  R  F   S  G  S  G   S  G  T   D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L  T  I  S   S  L  Q   P  E  D   F  A  T  Y   Y  C  S   Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H  V  P  L   T  F  G   Q  G  T   K  V  E  I   K  R  T   V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P  S  V  F   I  F  P   P  S  D   E  Q  L  K   S  G  T   A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L   N  N  F   Y  P  R   E  A  K  V   Q  W  K   V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S   G  N  S   Q  E  S   V  T  E  Q   D  S  K   D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S   S  T  L   T  L  S   K  A  D  Y   E  K  H   K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V   T  H  Q   G  L  S   S  P  V  T   K  S  F   N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218 E  C  O
```

FIG. 31B

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V19 Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVKQ**A**PGKGLEWVGYIDPSNGETTYNQKFKGRFT**L**SRDNSKNT**A**YLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

FIG. 31C

FIG. 32

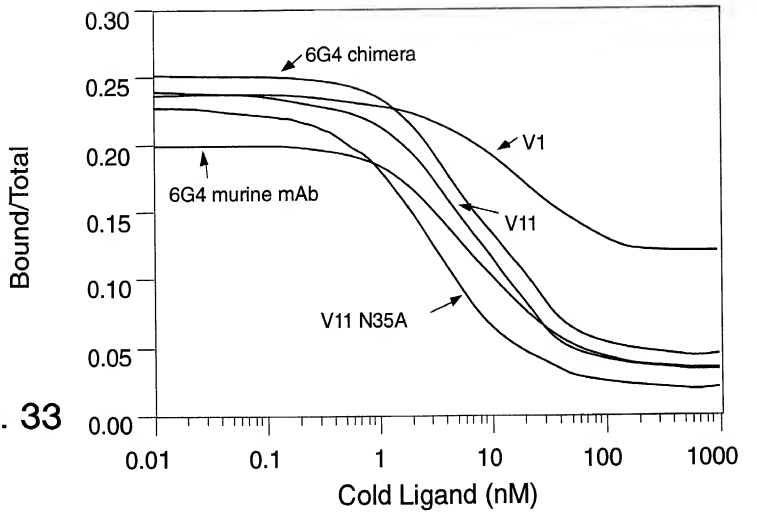

0.30
0.25
0.20
0.15
0.10
0.05
0.00
Bound/Total
6G4 chimera
V1
6G4 murine mAb
V11
V11 N35A
0.01
0.1
1
10
100
1000
Cold Ligand (nM)

FIG. 33

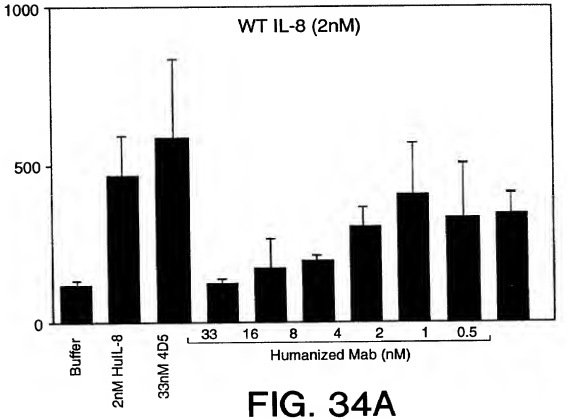


FIG. 34A

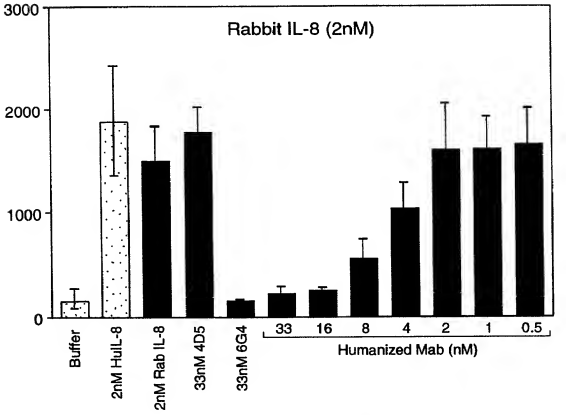


FIG. 34B

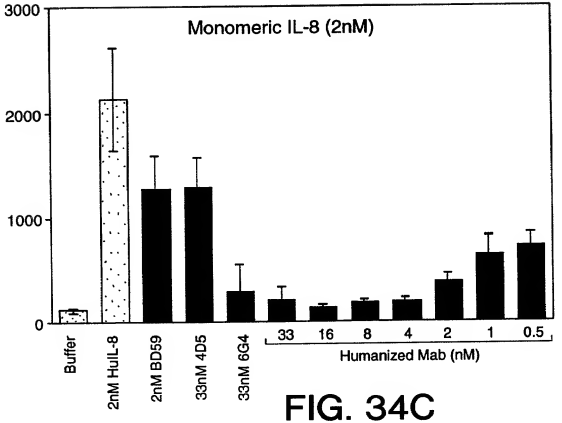


FIG. 34C

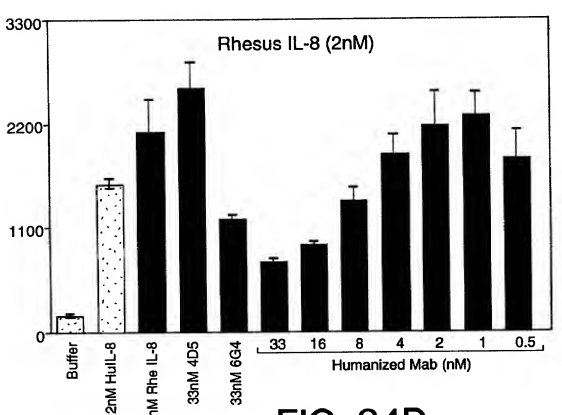


FIG. 34D

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Light Chain

MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGATY
LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST
HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

Amino Acid Sequence of the humanized anti-IL-8 6G4.2.5V11N35A Heavy Chain

MKKNIAFLLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH
WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY
CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHT

Amino Acid Sequence of the putative Pepsin Cleavage Site and GCN4 Leucine Zipper

CPPCPAPELLGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

FIG. 35

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23  M  K  K  N     I  A  F     L  L  A     S  M  F  V     F  S  I     A  T  N

 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3  A  Y  A  D     I  Q  M     T  Q  S     P  S  S  L     S  A  S     V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGCTACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACGATGCATA
 18  R  V  T  I     T  C  R  S  S  Q  S  L  V  H     G  I  G  A  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38  L  H  W  Y     Q  Q  K     P  G  K     A  P  K  L     L  I  Y     K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58  N  R  F  S     G  V  P     S  R  F     S  G  S  G     S  G  T     D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78  L  T  I  S     S  L  Q     P  E  D     F  A  T  Y     Y  C     S  Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98  H  V  P  L  T     F  G     Q  G  T     K  V  E  I     K  R  T     V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118  P  S  V  F     I  F  P     P  S  D     E  Q  L  K     S  G  T     A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138  V  C  L  L     N  N  F     Y  P  R     E  A  K  V     Q  W  K     V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158  A  L  Q  S     G  N  S     Q  E  S     V  T  E  Q     D  S  K     D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178  Y  S  L  S     S  T  L     T  L  S     K  A  D  Y     E  K  H     K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198  A  C  E  V     T  H  Q     G  L  S     S  P  V  T     K  S  F     N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218  E  C  O
```

FIG. 36

```
781 AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
    TTTTCCCATA GATCTCCAAC TCCACTAAAA TACTTTTTCT TATAGCGTAA AGAAGAACGT
 -1                                   M  K  K  N   I  A  F   L  L  A

841 TCTATGTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG AGGTTCAGCT AGTGCAGTCT
    AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
-11 S  M  F  V   F  S  I   A  T  N   A  Y  A  E   V  Q  L   V  Q  S

901 GGCGGTGGCC TGGTGCAGCC AGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
    CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
 8  G  G  G  L   V  Q  P   G  G  S   L  R  L  S   C  A  A   S  G  Y

961 TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
    AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTCCC GGACCTTACC
 28 S  F  S  S   H  Y  M   H  W  V   R  Q  A  P   G  K  G   L  E  W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
     CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
 48 V  G  Y  I   D  P  S   N  G  E   T  T  Y  N   Q  K  F   K  G  R

1081 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
     AAGTGAAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
 68 F  T  L  S   R  D  N   S  K  N   T  A  Y  L   Q  M  N   S  L  R

1141 GCTGAGGACA CTGCCGTCTA TTACTGTGCA AGAGGGGATT ATCGCTACAA TGGTGACTGG
     CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
 88  A  E  D  T   A  V  Y   Y  C  A   R  G  D  Y   R  Y  N   G  D  W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
     AAGAAGCTGC AGACCCCAGT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
 108 F  F  D  V   W  G  Q   G  T  L   V  T  V  S   S  A  S   T  K  G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
 128 P  S  V  F   P  L  A   P  S  S   K  S  T  S   G  G  T   A  A  L

1321 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
 148 G  C  L  V   K  D  Y   F  P  E   P  V  T  V   S  W  N   S  G  A

1381 CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
     GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
 168 L  T  S  G   V  H  T   F  P  A   V  L  Q  S   S  G  L   Y  S  L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
     TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC
 188 S  S  V  V   T  V  P   S  S  S   L  G  T  Q   T  Y  I   C  N  V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
     TTAGTGTTCG GGTCGTTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
 208 N  H  K  P   S  N  T   K  V  D   K  K  V  E   P  K  S   C  D  K

1561 ACTCACACAT GCCCGCCGTG CCCAGCACCA GAACTGCTGG GCGGCCGCAT GAAACAGCTA
     TGAGTGTGTA CGGGCGGCAC GGGTCGTGGT CTTGACGACC CGCCGGCGTA CTTTGTCGAT
 228 T  H  T  C   P  P  C   P  A  P   E  L  L  G   G  R  M   K  Q  L
```

FIG. 37A

```
1621 GAGGACAAGG TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA
     CTCCTGTTCC AGCTTCTCGA TGAGAGGTTC TTGATGGTGG ATCTCTTACT TCACCGTTCT
 248 E  D  K  V   E  E  L   L  S  K   N  Y  H  L   E  N  E   V  A  R

1681 CTCAAAAAGC TTGTCGGGGA GCGCTAA
     GAGTTTTTCG AACAGCCCCT CGCGATT
 268 L  K  K  L   V  G  E   R  O
```

FIG. 37B

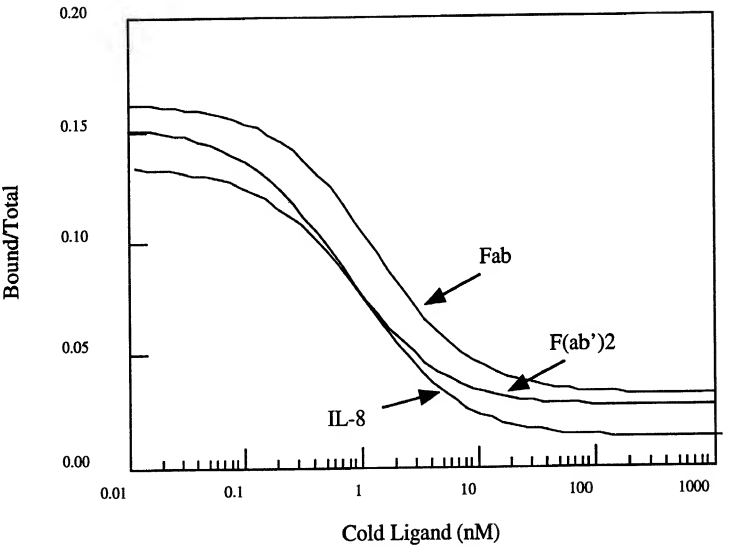

0.20
0.15
0.10
0.05
0.00
Bound/Total
Fab
F(ab')2
IL-8
0.01
0.1
1
10
100
1000
Cold Ligand (nM)

FIG. 38

FIG. 39

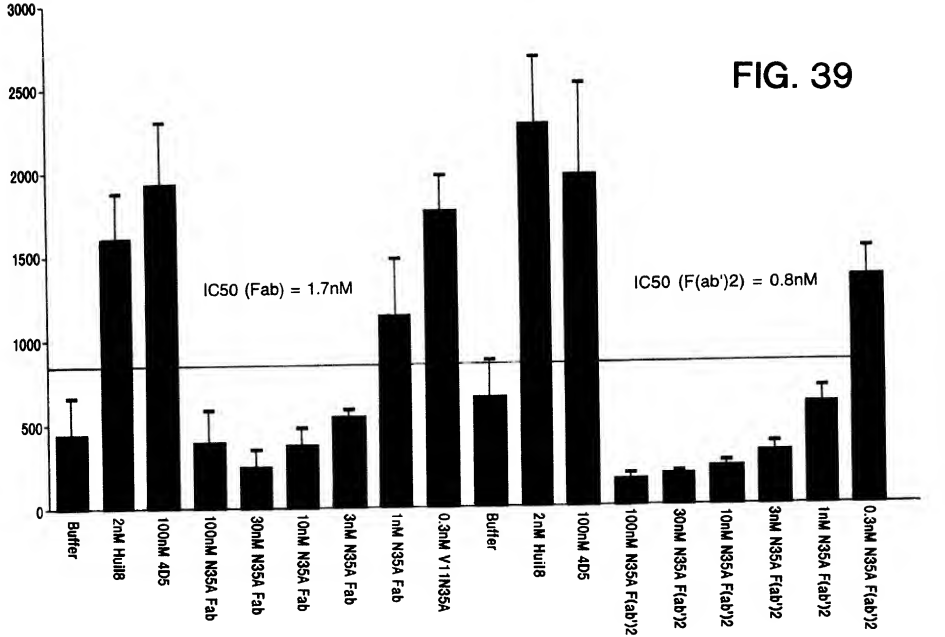
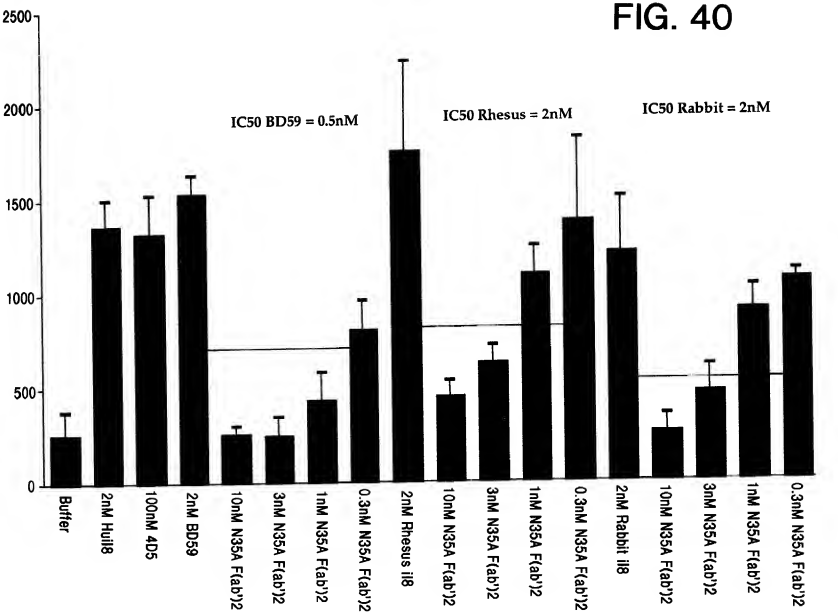

3000
2500
2000
1500
1000
500
0
IC50 (Fab) = 1.7nM
IC50 (F(ab')2) = 0.8nM
Buffer
2nM Huli8
100nM 4D5
100nM N35A Fab
30nM N35A Fab
10nM N35A Fab
3nM N35A Fab
1nM N35A Fab
0.3nM V11N35A
Buffer
2nM Huli8
100nM 4D5
100nM N35A F(ab')2
30nM N35A F(ab')2
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2

FIG. 40


2500
2000
1500
1000
500
0
IC50 BD59 = 0.5nM
IC50 Rhesus = 2nM
IC50 Rabbit = 2nM
Buffer
2nM Hul8
100nM 4D5
2nM BD59
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2
2nM Rhesus il8
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2
2nM Rabbit il8
10nM N35A F(ab')2
3nM N35A F(ab')2
1nM N35A F(ab')2
0.3nM N35A F(ab')2

ecoRI
apoI
pflMI
bslI
nlaIII
ddeI
bsrDI
aluI
hindIII
tru9I
mseI cac8I
pleI
mboII taqI
earI/ksp632I
mboII hinfI
1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTCTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT
CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA
bspMI
hinPI
hhaI/cfoI
mstI
aviII/fspI
aluI
hindIII
msII
maeIII bsrDI
hinPI
hhaI/cfoI
aciI
nspBII
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bclI[dam-] mnlI
101 GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGATTGAT CAGGTAGAGG
CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC
thaI
fnuDII/mvnI
fnu4HI
bsoFI
bbvI
maeII
fnu4HI bstUI snaBI
bsoFI bshl236I
bbvI hinPI bsaAI
aluI hhaI/cfoI
rsaI
hinPI
hhaI/cfoI mnlI
haeII csp6I
cac8I
sfaNI bsmI
mnlI
fokI
sfaNI
201 GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA
CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
ecoRI bsp1286
rmaI bsiHKAI
maeI bmyI
bfaI taqI
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
bsiEI ahdI/eam1105I
tru9I
mseI
aluI
pvuII
nspBII
maeIII bsmAI
tru9I
mseI
maeIII apoI banII
301 AAAAGTTAAT CTTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GAATTCGAGC
TTTTCAATTA GAAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT AAACATTGAT CTTAAGCTCG

FIG. 41A

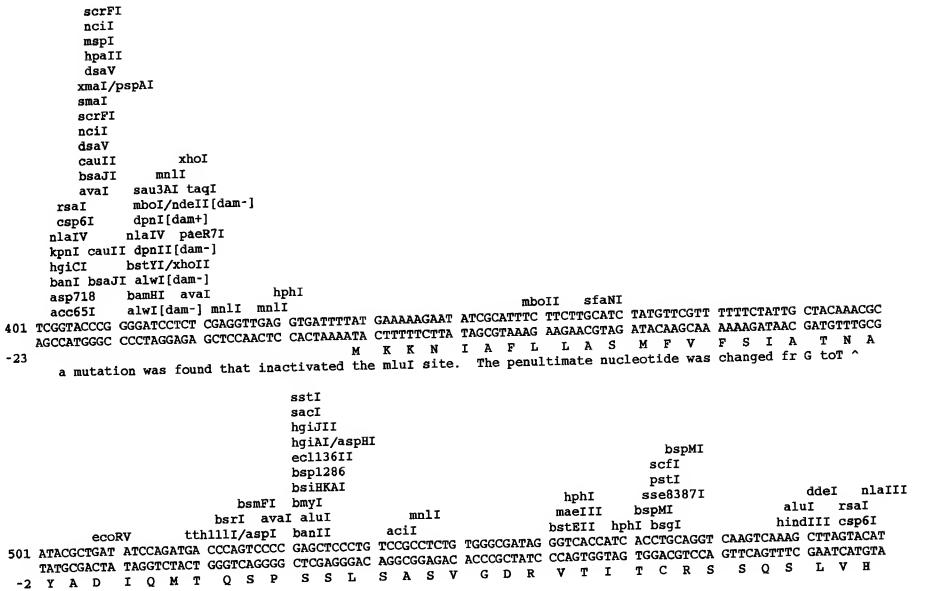

scrFI
nciI
mspI
hpaII
dsaV
xmaI/pspAI
smaI
scrFI
nciI
dsaV
cauII
xhoI
bsaJI
mnlI
avaI
sau3AI taqI
rsaI
mboI/ndeII[dam-]
csp6I
dpnI[dam+]
nlaIV
nlaIV paeR7I
kpnI cauII dpnII[dam-]
hgiCI
bstYI/xhoII
banI bsaJI alwI[dam-]
asp718
bamHI avaI
hphI
acc65I
alwI[dam-] mnlI mnlI
mboII sfaNI
401 TCGGTACCCG GGGATCCTCT CGAGGTTGAG GTGATTTTAT GAAAAAGAAT ATCGCATTTC TTCTTGCATC TATGTTCGTT TTTTCTATTG CTACAAACGC
AGCCATGGGC CCCTAGGAGA GCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCG
M K K N I A F L L A S M F V F S I A T N A
-23
a mutation was found that inactivated the mluI site. The penultimate nucleotide was changed fr G toT ^
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
bspMI
scfI
pstI
sse8387I
hphI
ddeI nlaIII
aluI rsaI
hindIII csp6I
bsmFI bmyI
bsrI avaI aluI
mnlI
maeIII bspMI
bstEII hphI bsgI
ecoRV
tth111I/aspI banII
acil
501 ATACGCTGAT ATCCAGATGA CCCAGTCCCC GAGCTCCCTG TCCGCCTCTG TGGGCGATAG GGTCACCATC ACCTGCAGGT CAAGTCAAAG CTTAGTACAT
TATGCGACTA TAGGTCTACT GGGTCAGGGG CTCGAGGGAC AGGCGGAGAC ACCCGCTATC CCAGTGGTAG TGGACGTCCA GTTCAGTTTC GAATCATGTA
-2 Y A D I Q M T Q S P S S L S A S V G D R V T I T C R S S Q S L V H

FIG. 41B

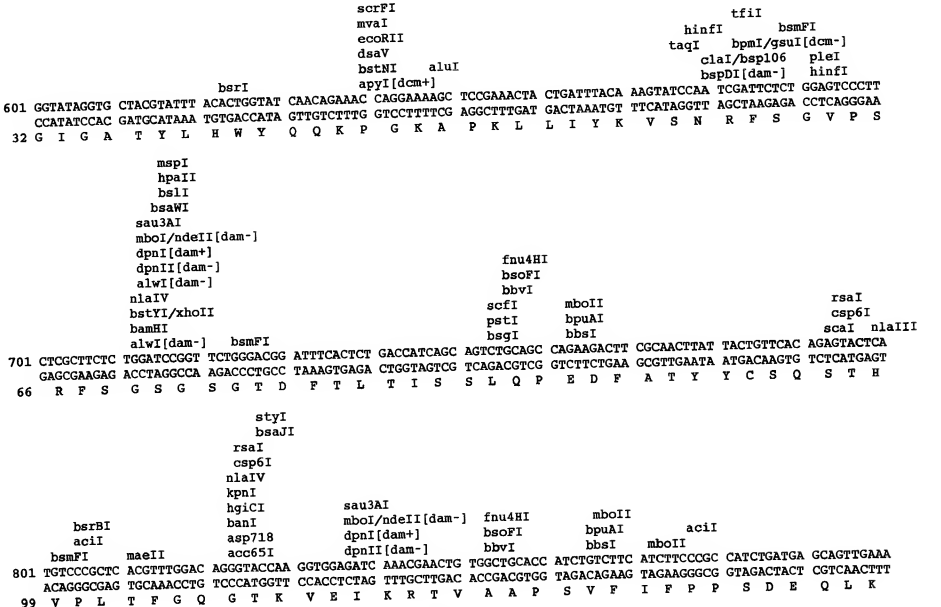
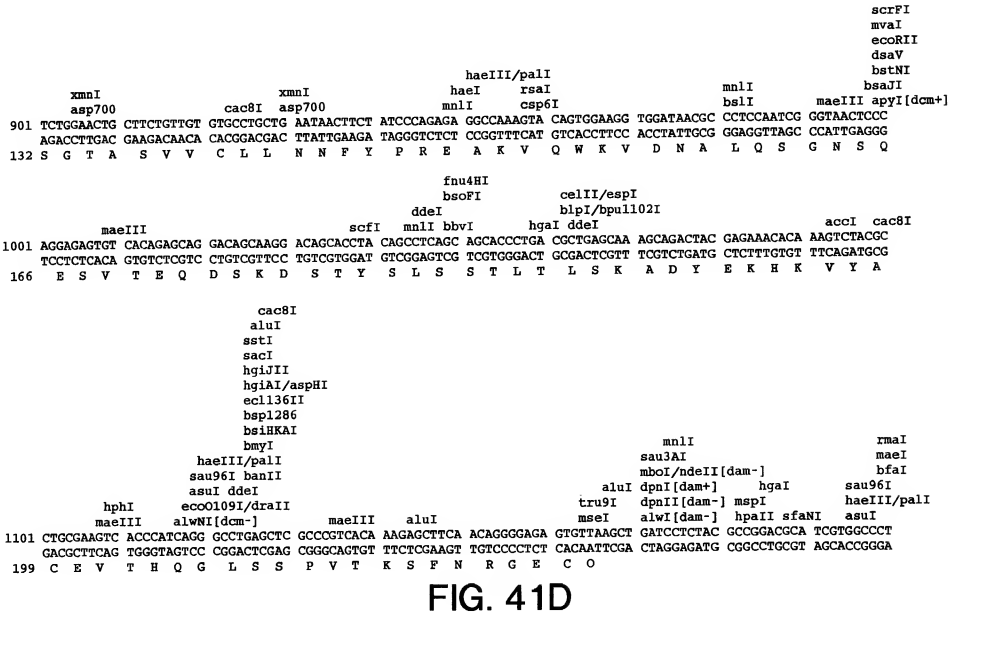

scrFI
mvaI
ecoRII
dsaV
bstNI aluI
apyI[dcm+]
tfiI
hinfI bsmFI
taqI bpmI/gsuI[dcm-]
claI/bsp106 pleI
bspDI[dam-] hinfI
bsrI
601 GGTATAGGTG CTACGTATTT ACACTGGTAT CAACAGAAAC CAGGAAAAGC TCCGAAACTA CTGATTTACA AAGTATCCAA TCGATTCTCT GGAGTCCCTT
CCATATCCAC GATGCATAAA TGTGACCATA GTTGTCTTTG GTCCTTTTCG AGGCTTTGAT GACTAAATGT TTCATAGGTT AGCTAAGAGA CCTCAGGGAA
32 G I G A T Y L H W Y Q Q K P G K A P K L L I Y K V S N R F S G V P S
mspI
hpaII
bslI
bsaWI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
nlaIV
bstYI/xhoII
bamHI
alwI[dam-] bsmFI
fnu4HI
bsoFI
bbvI
scfI mboII
pstI bpuAI
bsgI bbsI
rsaI
csp6I
scaI nlaIII
701 CTCGCTTCTC TGGATCCGGT TCTGGGACGG ATTTCACTCT GACCATCAGC AGTCTGCAGC CAGAAGACTT CGCAACTTAT TACTGTTCAC AGAGTACTCA
GAGCGAAGAG ACCTAGGCCA AGACCCTGCC TAAAGTGAGA CTGGTAGTCG TCAGACGTCG GTCTTCTGAA GCGTTGAATA ATGACAAGTG TCTCATGAGT
66 R F S G S G S G T D F T L T I S S L Q P E D F A T Y Y C S Q S T H
styI
bsaJI
rsaI
csp6I
nlaIV
kpnI
hgiCI
banI
asp718
acc65I
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
fnu4HI
bsoFI
bbvI
mboII
bpuAI
bbsI
aciI
mboII
bsrBI
aciI
bsmFI maeII
801 TGTCCCGCTC ACGTTTGGAC AGGGTACCAA GGTGGAGATC AAACGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC CATCTGATGA GCAGTTGAAA
ACAGGGCGAG TGCAAACCTG TCCCATGGTT CCACCTCTAG TTTGCTTGAC ACCGACGTGG TAGACAGAAG TAGAAGGGCG GTAGACTACT CGTCAACTTT
99 V P L T F G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K

FIG. 41C


scrFI
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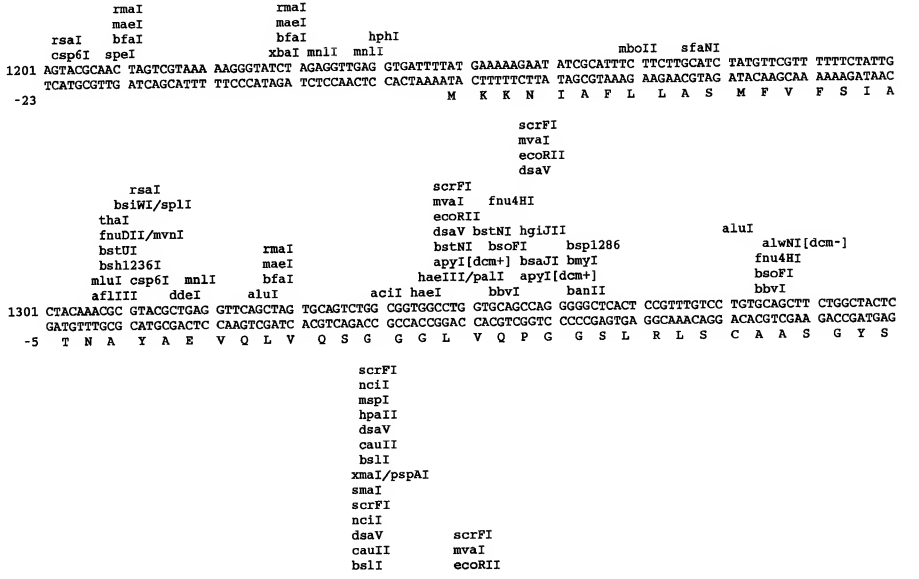
ecoRII
dsaV
bstNI
haeIII/palI
xmnI
haeI
rsaI
mnlI
bsaJI
xmnI
mnlI
bslI
maeIII apyI[dcm+]
asp700
cac8I asp700
csp6I
901 TCTGGAACTG CTTCTGTTGT GTGCCTGCTG AATAACTTCT ATCCCAGAGA GCCCAAAGTA CAGTGGAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC
AGACCTTGAC GAAGACAACA CACGGACGAC TTATTGAAGA TAGGGTCTCT CCGGTTTCAT GTCACCTTCC ACCTATTGCG GGAGGTTAGC CCATTGAGGG
132 S G T A S V V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q
fnu4HI
bsoFI
ddeI
celII/espI
blpI/bpul102I
maeIII
scfI mnlI bbvI
hgaI ddeI
accI cac8I
1001 AGGAGAGTGT CACAGAGCAG GACAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCTGA CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC
TCCTCTCACA GTGTCTCGTC CTGTCGTTCC TGTCGTGGAT GTCGGAGTCG TCGTGGGACT GCGACTCGTT TCGTCTGATG CTCTTTGTGT TTCAGATGCG
166 E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A
cac8I
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bspl286
bsiHKAI
bmyI
mnlI
rmaI
sau3AI
maeI
haeIII/palI
mboI/ndeII[dam-]
bfaI
sau96I banII
asuI ddeI
aluI dpnI[dam+]
hgaI
sau96I
hphI
eco0109I/draII
tru9I
dpnII[dam-] mspI
haeIII/palI
maeIII
alwNI[dcm-]
maeIII
aluI
mseI
alwI[dam-] hpaII sfaNI
asuI
1101 CTGCGAAGTC ACCCATCAGG GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAAGCT GATCCTCTAC GCCGGACGCA TCGTGGCCCT
GACGCTTCAG TGGGTAGTCC CGGACTCGAG CGGGCAGTGT TTCTCGAAGT TGTCCCCTCT CACAATTCGA CTAGGAGATG CGGCCTGCGT AGCACCGGGA
199 C E V T H Q G L S S P V T K S F N R G E C O

FIG. 41D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bfaI
hphI
rsaI
csp6I speI
xbaI mnlI mnlI
mboII sfaNI
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TCATGCGTTG ATCAGCATTT TTCCCATAGA TCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC
-23
M K K N I A F L L A S M F V F S I A
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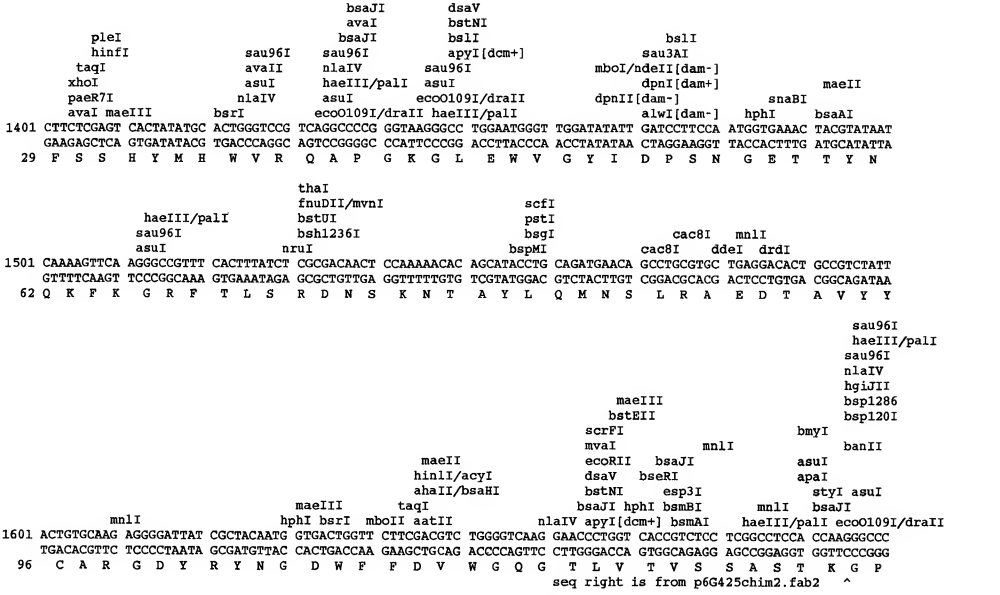
mvaI
ecoRII
dsaV
rsaI
bsiWI/splI
thaI
fnuDII/mvnI
bstUI
bsh1236I
mluI csp6I mnlI
aflIII ddeI
aluI
scrFI
mvaI fnu4HI
ecoRII
dsaV bstNI hgiJII
bstNI bsoFI bsp1286
apyI[dcm+] bsaJI bmyI
haeIII/palI apyI[dcm+]
aciI haeI bbvI banII
aluI
alwNI[dcm-]
fnu4HI
bsoFI
bbvI
1301 CTACAAACGC GTACGCTGAG GTTCAGCTAG TGCAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC TGTGCAGCTT CTGGCTACTC
GATGTTTGCG CATGCGACTC CAAGTCGATC ACGTCAGACC GCCACCGGAC CACGTCGGTC CCCCGAGTGA GGCAAACAGG ACACGTCGAA GACCGATGAG
-5 T N A Y A E V Q L V Q S G G G L V Q P G G S L R L S C A A S G Y S
scrFI
nciI
mspI
hpaII
dsaV
cauII
bslI
xmaI/pspAI
smaI
scrFI
nciI
dsaV scrFI
cauII mvaI
bslI ecoRII

FIG. 41E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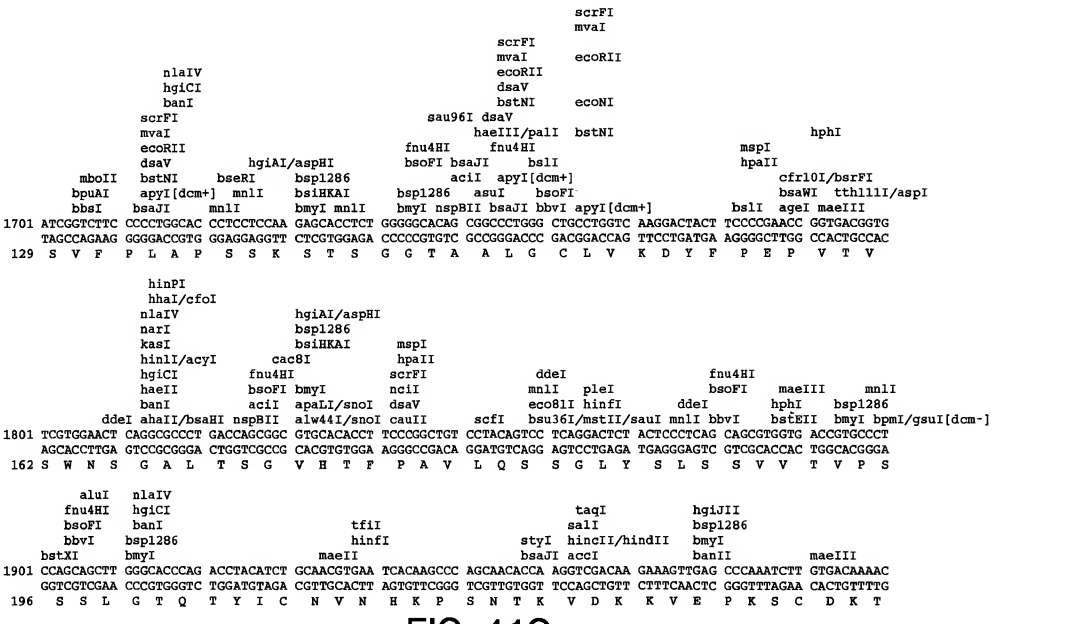
avaI bstNI
pleI bsaJI bslI bslI
hinfI sau96I sau96I apyI[dcm+] sau3AI
taqI avaII nlaIV sau96I mboI/ndeII[dam-]
xhoI asuI haeIII/palI asuI dpnI[dam+] maeII
paeR7I nlaIV asuI eco0109I/draII dpnII[dam-] snaBI
avaI maeIII bsrI eco0109I/draII haeIII/palI alwI[dam-] hphI bsaAI
1401 CTTCTCGAGT CACTATATGC ACTGGGTCCG TCAGGCCCCG GGTAAGGGCC TGGAATGGGT TGGATATATT GATCCTTCCA ATGGTGAAAC TACGTATAAT
GAAGAGCTCA GTGATATACG TGACCCAGGC AGTCCGGGGC CCATTCCCGG ACCTTACCCA ACCTATATAA CTAGGAAGGT TACCACTTTG ATGCATATTA
29 F S S H Y M H W V R Q A P G K G L E W V G Y I D P S N G E T T Y N
thaI
fnuDII/mvnI scfI
haeIII/palI bstUI pstI
sau96I bsh1236I bsgI cac8I mnlI
asuI nruI bspMI cac8I ddeI drdI
1501 CAAAAGTTCA AGGGCCGTTT CACTTTATCT CGCGACAACT CCAAAAACAC AGCATACCTG CAGATGAACA GCCTGCGTGC TGAGGACACT GCCGTCTATT
GTTTTCAAGT TCCCGGCAAA GTGAAATAGA GCGCTGTTGA GGTTTTTGTG TCGTATGGAC GTCTACTTGT CGGACGCACG ACTCCTGTGA CGGCAGATAA
62 Q K F K G R F T L S R D N S K N T A Y L Q M N S L R A E D T A V Y Y
sau96I
haeIII/palI
sau96I
nlaIV
hgiJII
maeIII bsp1286
bstEII bsp120I
scrFI bmyI
mvaI mnlI banII
maeII ecoRII bsaJI asuI
hinlI/acyI dsaV bseRI apaI
ahaII/bsaHI bstNI esp3I styI asuI
maeIII taqI bsaJI hphI bsmBI mnlI bsaJI
mnlI hphI bsrI mboII aatII nlaIV apyI[dcm+] bsmAI haeIII/palI eco0109I/draII
1601 ACTGTGCAAG AGGGGATTAT CGCTACAATG GTGACTGGTT CTTCGACGTC TGGGGTCAAG GAACCCTGGT CACCGTCTCC TCGGCCTCCA CCAAGGGCCC
TGACACGTTC TCCCCTAATA GCGATGTTAC CACTGACCAA GAAGCTGCAG ACCCCAGTTC CTTGGGACCA GTGGCAGAGG AGCCGGAGGT GGTTCCCGGG
96 C A R G D Y R Y N G D W F F D V W G Q G T L V T V S S A S T K G P
seq right is from p6G425chim2.fab2 ^

FIG. 41F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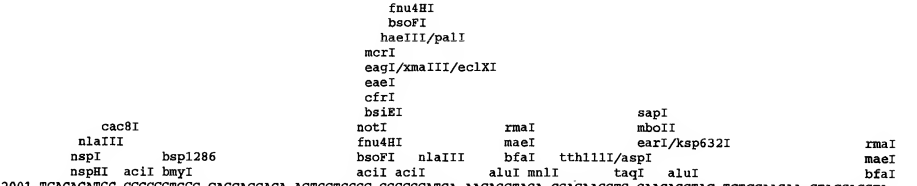
scrFI
mvaI ecoRII
nlaIV ecoRII
hgiCI dsaV
banI bstNI ecoNI
scrFI sau96I dsaV
mvaI haeIII/palI bstNI hphI
ecoRII fnu4HI fnu4HI mspI
dsaV hgiAI/aspHI bsoFI bsaJI bslI hpaII
mboII bstNI bseRI bsp1286 aciI apyI[dcm+] cfr10I/bsrFI
bpuAI apyI[dcm+] mnlI bsiHKAI bsp1286 asuI bsoFI bsaWI tth111I/aspI
bbsI bsaJI mnlI bmyI mnlI bmyI nspBII bsaJI bbvI apyI[dcm+] bslI ageI maeIII
1701 ATCGGTCTTC CCCCTGGCAC CCTCCTCCAA GAGCACCTCT GGGGGCACAG CGGCCCTGGG CTGCCTGGTC AAGGACTACT TCCCCGAACC GGTGACGGTG
TAGCCAGAAG GGGGACCGTG GGAGGAGGTT CTCGTGGAGA CCCCCGTGTC GCCGGGACCC GACGGACCAG TTCCTGATGA AGGGGCTTGG CCACTGCCAC
129 S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V
hinPI
hhaI/cfoI
nlaIV hgiAI/aspHI
narI bsp1286
kasI bsiHKAI mspI
hinlI/acyI cac8I hpaII
hgiCI fnu4HI scrFI ddeI fnu4HI
haeII bsoFI bmyI nciI mnlI pleI bsoFI maeIII mnlI
banI aciI apaLI/snoI dsaV eco81I hinfI ddeI hphI bsp1286
ddeI ahaII/bsaHI nspBII alw44I/snoI cauII scfI bsu36I/mstII/sauI mnlI bbvI bstEII bmyI bpmI/gsuI[dcm-]
1801 TCGTGGAACT CAGGCGCCCT GACCAGCGGC GTGCACACCT TCCCGGCTGT CCTACAGTCC TCAGGACTCT ACTCCCTCAG CAGCGTGGTG ACCGTGCCCT
AGCACCTTGA GTCCGCGGGA CTGGTCGCCG CACGTGTGGA AGGGCCGACA GGATGTCAGG AGTCCTGAGA TGAGGGAGTC GTCGCACCAC TGGCACGGGA
162 S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S
aluI nlaIV
fnu4HI hgiCI taqI hgiJII
bsoFI banI tfiI salI bsp1286
bbvI bsp1286 hinfI styI hincII/hindII bmyI
bstXI bmyI maeII bsaJI accI banII maeIII
1901 CCAGCAGCTT GGGCACCCAG ACCTACATCT GCAACGTGAA TCACAAGCCC AGCAACACCA AGGTCGACAA GAAAGTTGAG CCCAAATCTT GTGACAAAAC
GGTCGTCGAA CCCGTGGGTC TGGATGTAGA CGTTGCACTT AGTGTTCGGG TCGTTGTGGT TCCAGCTGTT CTTTCAACTC GGGTTTAGAA CACTGTTTTG
196 S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C D K T

FIG. 41G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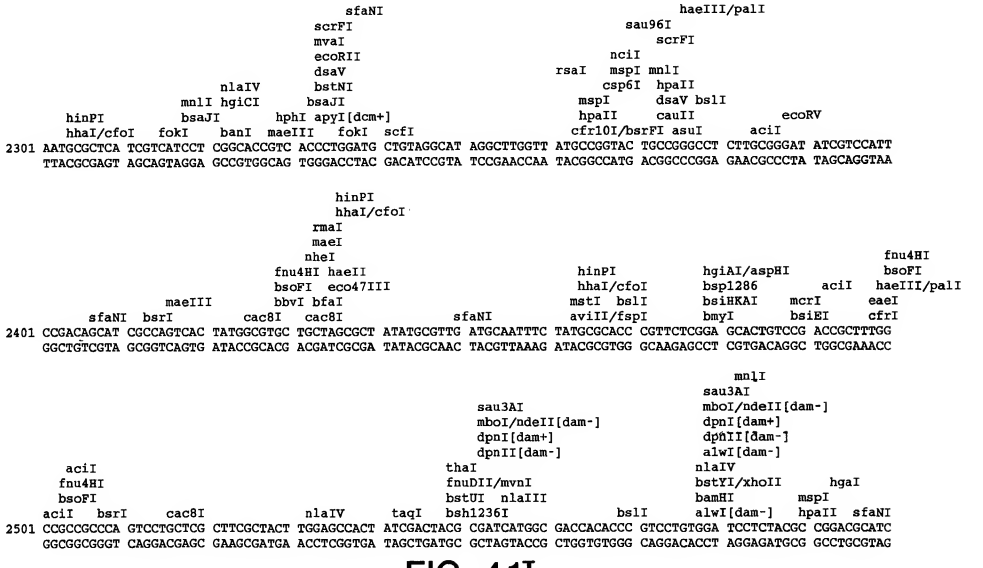
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
bsiEI
sapI
cac8I
notI
rmaI
mboII
nlaIII
fnu4HI
maeI
earI/ksp632I
rmaI
nspI
bsp1286
bsoFI
nlaIII
bfaI
tth111I/aspI
maeI
nspHI
aciI bmyI
aciI aciI
aluI mnlI
taqI
aluI
bfaI
2001 TCACACATGC CCGCCGTGCC CAGCACCAGA ACTGCTGGGC GGCCGCATGA AACAGCTAGA GGACAAGGTC GAAGAGCTAC TCTCCAAGAA CTACCACCTA
AGTGTGTACG GGCGGCACGG GTCGTGGTCT TGACGACCCG CCGGCGTACT TTGTCGATCT CCTGTTCCAG CTTCTCGATG AGAGGTTCTT GATGGTGGAT
229 H T C P P C P A P E L L G G R M K Q L E D K V E E L L S K N Y H L
^junction between antibody and leucine zipper
scrFI
sphI
nciI
ddeI nlaIII
mspI
celII/espI
rmaI
hpaII
blpI/bpu1102I
maeI
dsaV
hinPI nspI
bfaI bsmFI
cauII
hhaI/cfoI
sau96I pleI
aciI
pleI
aluI
haeII nspHI
haeIII/palI
fnu4HI
hinfI
hindIII
eco47III cac8I
asuI hinfI
bsoFI
2101 GAGAATGAAG TGGCAAGACT CAAAAAGCTT GTCGGGGAGC GCTAAGCATG CGACGGCCCT AGAGTCCCTA ACGCTCGGTT GCCGCCGGGC GTTTTTTATT
CTCTTACTTC ACCGTTCTGA GTTTTTCGAA CAGCCCCTCG CGATTCGTAC GCTGCCGGGA TCTCAGGGAT TGCGAGCCAA CGGCGGCCCG CAAAAAATAA
262 E N E V A R L K K L V G E R O
tru9I
aluI
mseI
taqI hindIII
nlaIV
hpaI nlaIII
claI/bspl06 tru9I
tru9I
hgiCI
hincII/hindII aluI
bspDI[dam-] mseI aciI
mseI
banI
2201 GTTAACTCAT GTTTGACAGC TTATCATCGA TAAGCTTTAA TGCGGTAGTT TATCACAGTT AAATTGCTAA CGCAGTCAGG CACCGTGTAT GAAATCTAAC
CAATTGAGTA CAAACTGTCG AATAGTAGCT ATTCGAAATT ACGCCATCAA ATAGTGTCAA TTTAACGATT GCGTCAGTCC GTGGCACATA CTTTAGATTG

FIG. 41H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mvaI
ecoRII
dsaV
nlaIV
bstNI
mnlI hgiCI
bsaJI
hinPI
bsaJI
hphI apyI[dcm+]
hhaI/cfoI
fokI
banI
maeIII
fokI scfI
2301 AATGCGCTCA TCGTCATCCT CGGCACCGTC ACCCTGGATG CTGTAGGCAT AGGCTTGGTT ATGCCGGTAC TGCCGGGCCT CTTGCGGGAT ATCGTCCATT
TTACGCGAGT AGCAGTAGGA GCCGTGGCAG TGGGACCTAC GACATCCGTA TCCGAACCAA TACGGCCATG ACGGCCCGGA GAACGCCCTA TAGCAGGTAA
haeIII/palI
sau96I
scrFI
nciI
rsaI mspI mnlI
csp6I hpaII
mspI dsaV bslI
hpaII cauII ecoRV
cfr10I/bsrFI asuI aciI
hinPI
hhaI/cfoI
rmaI
maeI
nheI
fnu4HI haeII
bsoFI eco47III
maeIII bbvI bfaI
sfaNI bsrI cac8I cac8I sfaNI
2401 CCGACACGAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT ATATGCGTTG ATGCAATTTC TATGCGCACC CGTTCTCGGA GCACTGTCCG ACCGCTTTGG
GGCTGTCGTA GCGGTCAGTG ATACCGCACG ACGATCGCGA TATACGCAAC TACGTTAAAG ATACGCGTGG GCAAGAGCCT CGTGACAGGC TGGCGAAACC
hinPI
hhaI/cfoI
bsp1286
mstI bslI
aviII/fspI
hgiAI/aspHI
bsiHKAI
bmyI
fnu4HI
bsoFI
aciI haeIII/palI
mcrI eaeI
bsiEI cfrI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
thaI
fnuDII/mvnI
bstUI nlaIII
bsh1236I bslI
mnlI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
nlaIV
bstYI/xhoII hgaI
bamHI mspI
alwI[dam-] hpaII sfaNI
aciI
fnu4HI
bsoFI
aciI bsrI cac8I nlaIV taqI
2501 CCGCCGCCCA GTCCTGCTCG CTTCGCTACT TGGAGCCACT ATCGACTACG CGATCATGGC GACCACACCC GTCCTGTGGA TCCTCTACGC CGGACGCATC
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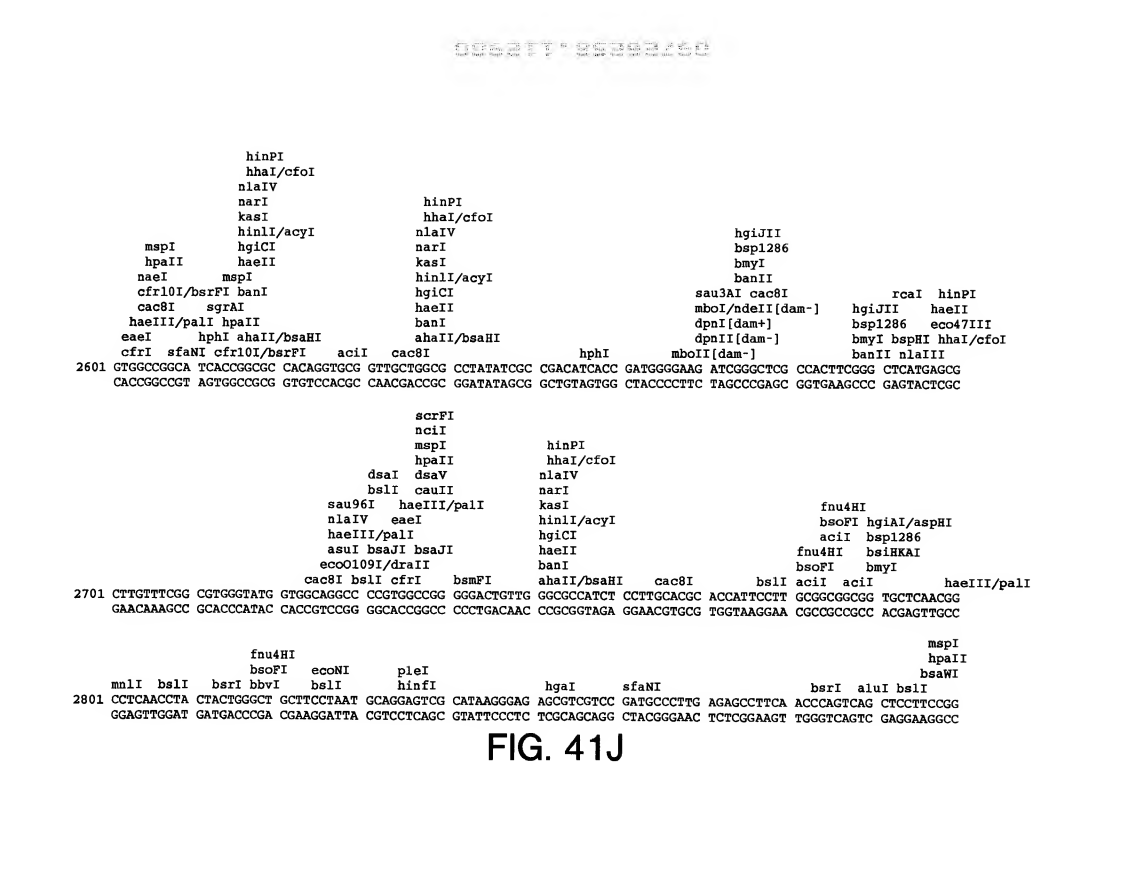
FIG. 41I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nlaIV
narI
kasI
hinlI/acyI
mspI
hgiCI
hpaII
haeII
naeI
mspI
cfr10I/bsrFI banI
cac8I sgrAI
haeIII/palI hpaII
eaeI hphI ahaII/bsaHI
cfrI sfaNI cfr10I/bsrFI aciI cac8I
2601 GTGGCCGGCA TCACCGGCGC CACAGGTGCG GTTGCTGGCG CCTATATCGC CGACATCACC GATGGGGAAG ATCGGGCTCG CCACTTCGGG CTCATGAGCG
CACCGGCCGT AGTGGCCGCG GTGTCCACGC CAACGACCGC GGATATAGCG GCTGTAGTGG CTACCCCTTC TAGCCCGAGC GGTGAAGCCC GAGTACTCGC
hinPI
hhaI/cfoI
nlaIV
narI
hgiJII
bsp1286
bmyI
banII
sau3AI cac8I
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
mboII[dam-]
rcaI hinPI
hgiJII haeII
bsp1286 eco47III
bmyI bspHI hhaI/cfoI
banII nlaIII
scrFI
nciI
mspI
hpaII
dsaI dsaV
bslI cauII
sau96I haeIII/palI
nlaIV eaeI
haeIII/palI
asuI bsaJI bsaJI
eco0109I/draII
cac8I bslI cfrI bsmFI
hinPI
hhaI/cfoI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII
banI
ahaII/bsaHI cac8I
fnu4HI
bsoFI hgiAI/aspHI
aciI bsp1286
fnu4HI bsiHKAI
bsoFI bmyI
bslI aciI aciI haeIII/palI
2701 CTTGTTTCGG CGTGGGTATG GTGGCAGGCC CCGTGGCCGG GGGACTGTTG GGCGCCATCT CCTTGCACGC ACCATTCCTT GCGGCGGCGG TGCTCAACGG
GAACAAAGCC GCACCCATAC CACCGTCCGG GGCACCGGCC CCCTGACAAC CCGCGGTAGA GGAACGTGCG TGGTAAGGAA CGCCGCCGCC ACGAGTTGCC
fnu4HI
bsoFI ecoNI
mnlI bslI bsrI bbvI bslI
pleI
hinfI
2801 CCTCAACCTA CTACTGGGCT GCTTCCTAAT GCAGGAGTCG CATAAGGGAG AGCGTCGTCC GATGCCCTTG AGAGCCTTCA ACCCAGTCAG CTCCTTCCGG
GGAGTTGGAT GATGACCCGA CGAAGGATTA CGTCCTCAGC GTATTCCCTC TCGCAGCAGG CTACGGGAAC TCTCGGAAGT TGGGTCAGTC GAGGAAGGCC
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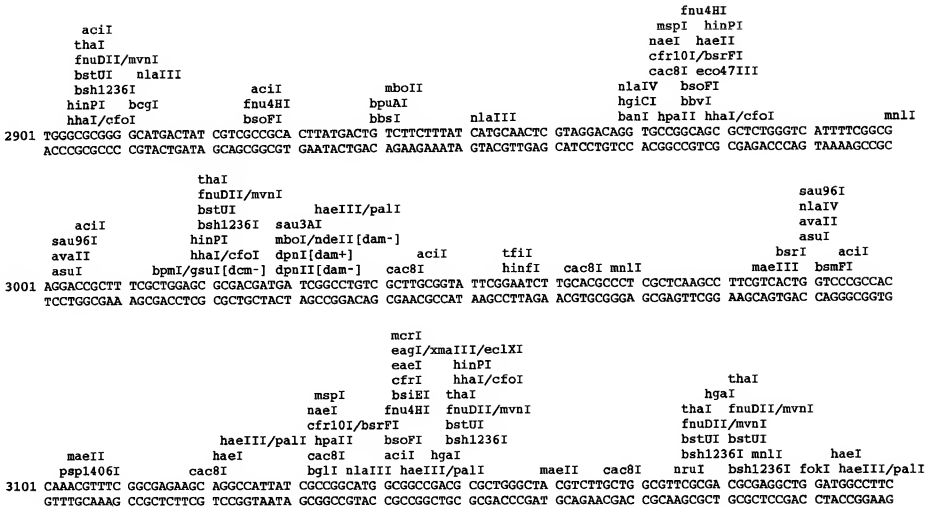
mspI
hpaII
bsaWI
bsrI aluI bslI

FIG. 41J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fnuDII/mvnI
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hhaI/cfoI
aciI
fnu4HI
bsoFI
mboII
bpuAI
bbsI
nlaIII
fnu4HI
mspI hinPI
naeI haeII
cfr10I/bsrFI
cac8I eco47III
nlaIV bsoFI
hgiCI bbvI
banI hpaII hhaI/cfoI
mnlI
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ACCCGCGCCC CGTACTGATA GCAGCGGCGT GAATACTGAC AGAAGAAATA GTACGTTGAG CATCCTGTCC ACGGCCGTCG CGAGACCCAG TAAAAGCCGC
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fnuDII/mvnI
bstUI
haeIII/palI
sau96I
nlaIV
avaII
asuI
aciI
bsh1236I
sau3AI
sau96I
hinPI
mboI/ndeII[dam-]
avaII
hhaI/cfoI
dpnI[dam+]
aciI
tfiI
bsrI aciI
asuI
bpmI/gsuI[dcm-]
dpnII[dam-]
cac8I
hinfI
cac8I mnlI
maeIII bsmPI
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TCCTGGCGAA AGCGACCTCG CGCTGCTACT AGCCGGACAG CGAACGCCAT AAGCCTTAGA ACGTGCGGGA GCGAGTTCGG AAGCAGTGAC CAGGGCGGTG
mcrI
eagI/xmaIII/eclXI
eaeI hinPI
cfrI hhaI/cfoI
thaI
mspI
naeI
cfr10I/bsrFI
bsiEI thaI
fnu4HI fnuDII/mvnI
bstUI
hgaI
thaI fnuDII/mvnI
fnuDII/mvnI
bstUI bstUI
haeIII/palI hpaII
bsoFI bsh1236I
bsh1236I mnlI
maeII
haeI
cac8I
aciI hgaI
haeI
psp1406I
cac8I
bglI nlaIII haeIII/palI
maeII cac8I
nruI bsh1236I fokI haeIII/palI
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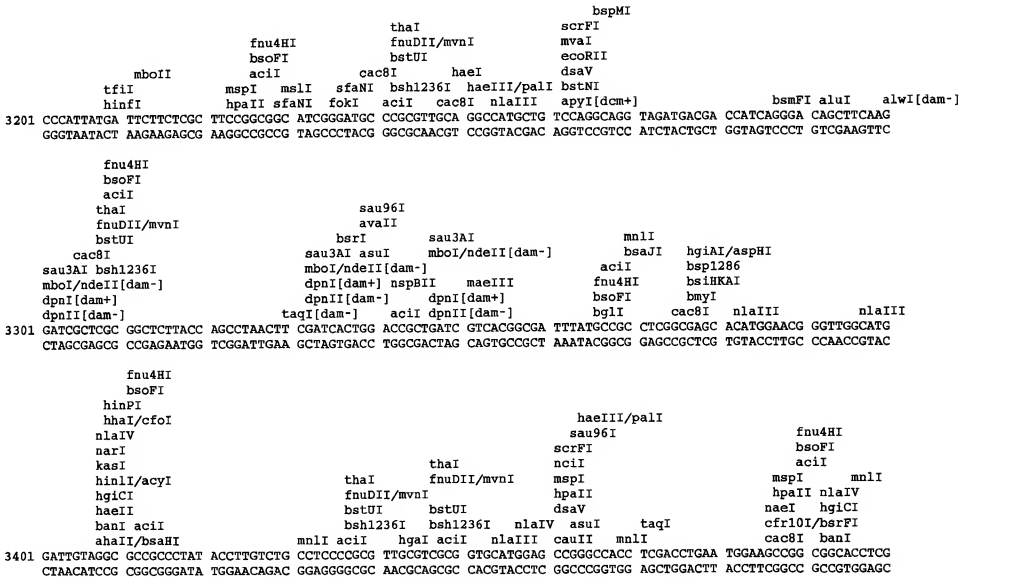
FIG. 41K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mboII
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cac8I
haeI
dsaV
tfiI
mspI
mslI
sfaNI
bsh1236I
haeIII/palI
bstNI
hinfI
hpaII
sfaNI
fokI
aciI
cac8I
nlaIII
apyI[dcm+]
bsmFI
aluI
alwI[dam-]
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GGGTAATACT AAGAAGAGCG AAGGCCGCCG TAGCCCTACG GGCGCAACGT CCGGTACGAC AGGTCCGTCC ATCTACTGCT GGTAGTCCCT GTCGAAGTTC
fnu4HI
bsoFI
aciI
thaI
sau96I
fnuDII/mvnI
avaII
bstUI
bsrI
sau3AI
mnlI
cac8I
sau3AI
asuI
mboI/ndeII[dam-]
bsaJI
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sau3AI
bsh1236I
mboI/ndeII[dam-]
aciI
bsp1286
mboI/ndeII[dam-]
dpnI[dam+]
nspBII
maeIII
fnu4HI
bsiHKAI
dpnI[dam+]
dpnII[dam-]
dpnI[dam+]
bsoFI
bmyI
dpnII[dam-]
taqI[dam-]
aciI
dpnII[dam-]
bglI
cac8I
nlaIII
nlaIII
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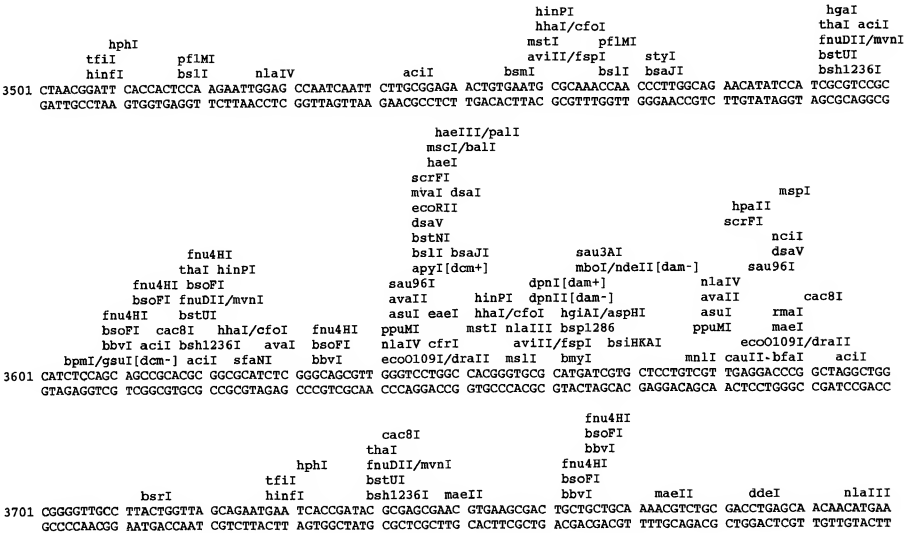
CTAGCGAGCG CCGAGAATGG TCGGATTGAA GCTAGTGACC TGGCGACTAG CAGTGCCGCT AAATACGGCG GAGCCGCTCG TGTACCTTGC CCAACCGTAC
fnu4HI
bsoFI
hinPI
haeIII/palI
hhaI/cfoI
sau96I
fnu4HI
nlaIV
scrFI
bsoFI
narI
thaI
nciI
aciI
kasI
thaI
fnuDII/mvnI
mspI
mspI
mnlI
hinlI/acyI
fnuDII/mvnI
hpaII
hpaII
nlaIV
hgiCI
bstUI
bstUI
dsaV
naeI
hgiCI
haeII
bsh1236I
bsh1236I
nlaIV
asuI
taqI
cfr10I/bsrFI
banI
aciI
ahaII/bsaHI
mnlI
aciI
hgaI
aciI
nlaIII
cauII
mnlI
cac8I
banI
3401 GATTGTAGGC GCCGCCCTAT ACCTTGTCTG CCTCCCCGCG TTGCGTCGCG GTGCATGGAG CCGGGCCACC TCGACCTGAA TGGAAGCCGG CGGCACCTCG
CTAACATCCG CGGCGGGATA TGGAACAGAC GGAGGGGCGC AACGCAGCGC CACGTACCTC GGCCCGGTGG AGCTGGACTT ACCTTCGGCC GCCGTGGAGC

FIG. 41L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hgaI
thaI aciI
hphI
tfiI
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mstI
pflMI
aviII/fspI
bsmI
bslI
styI
bsaJI
fnuDII/mvnI
bstUI
bsh1236I
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haeIII/palI
mscI/ball
haeI
scrFI
mvaI dsaI
mspI
ecoRII
hpaII
dsaV
scrFI
bstNI
nciI
fnu4HI
bslI bsaJI
sau3AI
dsaV
thaI hinPI
apyI[dcm+]
mboI/ndeII[dam-]
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fnu4HI bsoFI
sau96I
dpnI[dam+]
nlaIV
bsoFI fnuDII/mvnI
avaII
hinPI
dpnII[dam-]
avaII
cac8I
fnu4HI
bstUI
asuI eaeI
hhaI/cfoI
hgiAI/aspHI
asuI
rmaI
bsoFI
cac8I
hhaI/cfoI
fnu4HI
ppuMI
mstI nlaIII bsp1286
ppuMI
maeI
bbvI aciI bsh1236I
avaI
bsoFI
nlaIV cfrI
aviII/fspI
bsiHKAI
eco0109I/draII
bpmI/gsuI[dcm-] aciI
sfaNI
bbvI
eco0109I/draII
msII
bmyI
mnlI cauII-bfaI
aciI
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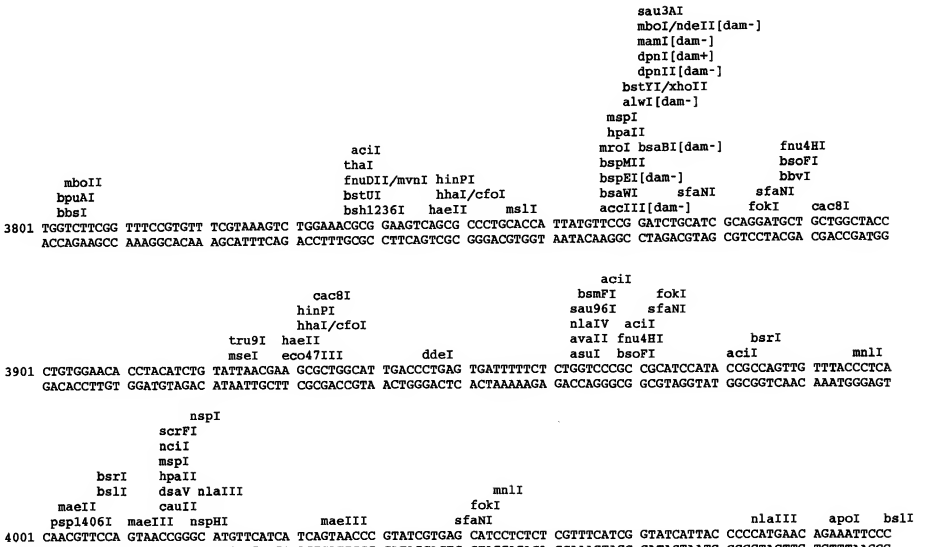
GTAGAGGTCG TCGGCGTGCG CCGCGTAGAG CCCGTCGCAA CCCAGGACCG GTGCCCACGC GTACTAGCAC GAGGACAGCA ACTCCTGGGC CGATCCGACC
fnu4HI
bsoFI
cac8I
bbvI
thaI
hphI
fnuDII/mvnI
fnu4HI
tfiI
bstUI
bsoFI
bsrI
hinfI
bsh1236I
maeII
bbvI
maeII
ddeI
nlaIII
3701 CGGGGTTGCC TTACTGGTTA GCAGAATGAA TCACCGATAC GCGAGCGAAC GTGAAGCGAC TGCTGCTGCA AAACGTCTGC GACCTGAGCA ACAACATGAA
GCCCCAACGG AATGACCAAT CGTCTTACTT AGTGGCTATG CGCTCGCTTG CACTTCGCTG ACGACGACGT TTTGCAGACG CTGGACTCGT TGTTGTACTT

FIG. 41M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alwI[dam-]
mspI
hpaII
aciI
thaI
fnuDII/mvnI hinPI
bstUI hhaI/cfoI
bsh1236I haeII msII
mroI bsaBI[dam-]
bspMII
bspEI[dam-]
bsaWI sfaNI
accIII[dam-]
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bsoFI
bbvI
sfaNI
fokI
cac8I
mboII
bpuAI
bbsI
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ACCAGAAGCC AAAGGCACAA AGCATTTCAG ACCTTTGCGC CTTCAGTCGC GGGACGTGGT AATACAAGGC CTAGACGTAG CGTCCTACGA CGACCGATGG
aciI
bsmFI fokI
sau96I sfaNI
nlaIV aciI
avaII fnu4HI
asuI bsoFI
bsrI
aciI
mnlI
cac8I
hinPI
hhaI/cfoI
tru9I haeII
mseI eco47III ddeI
3901 CTGTGGAACA CCTACATCTG TATTAACGAA GCGCTGGCAT TGACCCTGAG TGATTTTTCT CTGGTCCCGC CGCATCCATA CCGCCAGTTG TTTACCCTCA
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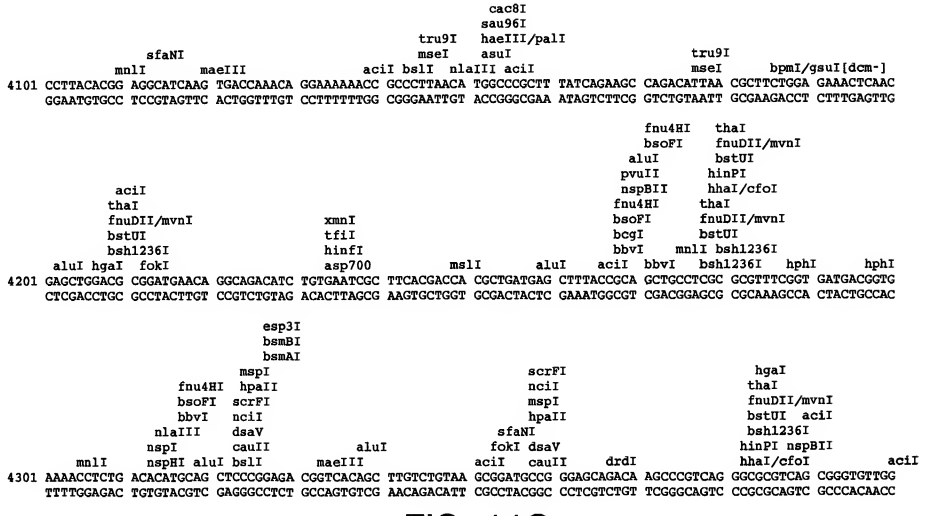
scrFI
nciI
mspI
bsrI hpaII
bslI dsaV nlaIII
mnlI
maeII cauII
fokI
psp1406I maeIII nspHI maeIII sfaNI
nlaIII apoI bslI
4001 CAACGTTCCA GTAACCGGGC ATGTTCATCA TCAGTAACCC GTATCGTGAG CATCCTCTCT CGTTTCATCG GTATCATTAC CCCCATGAAC AGAAATTCCC
GTTGCAAGGT CATTGGCCCG TACAAGTAGT AGTCATTGGG CATAGCACTC GTAGGAGAGA GCAAAGTAGC CATAGTAATG GGGGTACTTG TCTTTAAGGG

FIG. 41N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sau96I
tru9I haeIII/palI
sfaNI mseI asuI tru9I
mnlI maeIII aciI bslI nlaIII aciI mseI bpmI/gsuI[dcm-]
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GGAATGTGCC TCCGTAGTTC ACTGGTTTGT CCTTTTTTGG CGGGAATTGT ACCGGGCGAA ATAGTCTTCG GTCTGTAATT GCGAAGACCT CTTTGAGTTG
fnu4HI thaI
bsoFI fnuDII/mvnI
aluI bstUI
pvuII hinPI
aciI nspBII hhaI/cfoI
thaI fnu4HI thaI
fnuDII/mvnI xmnI bsoFI fnuDII/mvnI
bstUI tfiI bcgI bstUI
bsh1236I hinfI bbvI mnlI bsh1236I
aluI hgaI fokI asp700 mslI aluI aciI bbvI bsh1236I hphI hphI
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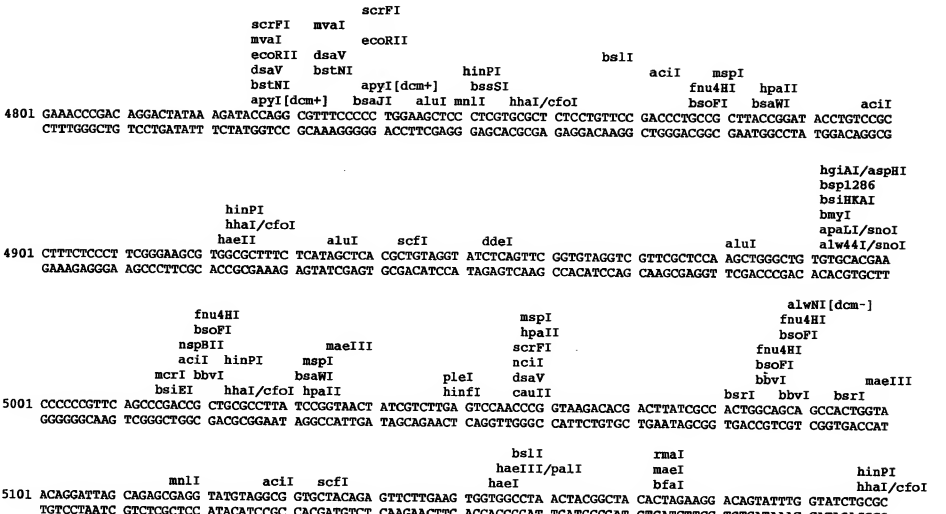
CTCGACCTGC GCCTACTTGT CCGTCTGTAG ACACTTAGCG AAGTGCTGGT GCGACTACTC GAAATGGCGT CGACGGAGCG CGCAAAGCCA CTACTGCCAC
esp3I
bsmBI
bsmAI
mspI scrFI hgaI
fnu4HI hpaII nciI thaI
bsoFI scrFI mspI fnuDII/mvnI
bbvI nciI hpaII bstUI aciI
nlaIII dsaV sfaNI bsh1236I
nspI cauII aluI fokI dsaV hinPI nspBII
mnlI nspHI aluI bslI maeIII aciI cauII drdI hhaI/cfoI aciI
4301 AAAACCTCTG ACACATGCAG CTCCCGGAGA CGGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG GGCGCGTCAG CGGGTGTTGG
TTTTGGAGAC TGTGTACGTC GAGGGCCTCT GCCAGTGTCG AACAGACATT CGCCTACGGC CCTCGTCTGT TCGGGCAGTC CCGCGCAGTC GCCCACAACC

FIG. 41O

hgiAI/aspHI
fnu4HI
bsp1286
bsoFI
maeII
sfaNI
bsiHKAI
bbvI
maeIII
fnu4HI
ddeI
bmyI ndeI
hinPI nlaIII bsrI bsaAI
bst1107I tru9I
bsoFI
rsaI
apaLI/snoI
hhaI/cfoI tth111I/aspI
aciI accI bsrI mseI
aciI
csp6I
alw44I/snoI
4401 CGGGTGTCGG GGCGCAGCCA TGACCCAGTC ACGTAGCGAT AGCGGAGTGT ATACTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC
GCCCACAGCC CCGCGTCGGT ACTGGGTCAG TGCATCGCTA TCGCCTCACA TATGACCGAA TTGATACGCC GTAGTCTCGT CTAACATGAC TCTCACGTGG
mboII
earI/ksp632I
hinPI
sapI
hhaI/cfoI
hinPI
fnu4HI
sfaNI
hhaI/cfoI
pleI bsoFI
mcrI
aciI
aciI
sfaNI
aciI
haeII
aciI mnlI
hinfI bbvI
bsiEI
4501 ATATGCGGTG TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG
TATACGCCAC ACTTTATGGC GTGTCTACGC ATTCCTCTTT TATGGCGTAG TCCGCGAGAA GGCGAAGGAG CGAGTGACTG AGCGACGCGA GCCAGCAAGC
fnu4HI
bsoFI
aciI
nlaIII
bslI
fnu4HI
aciI
nspI
cac8I
bsoFI bsrBI
tfiI
nspHI
haeIII/palI
bbvI cac8I
aluI
aciI
hinfI
aflIII
haeI
4601 GCTGCGGCGA GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA
CGACGCCGCT CGCCATAGTC GAGTGAGTTT CCGCCATTAT GCCAATAGGT GTCTTAGTCC CCTATTGCGT CCTTTCTTGT ACACTCGTTT TCCGGTCGTT
scrFI
thaI
mvaI
fnuDII/mvnI
ecoRII
bstUI
dsaV
bsh1236I
bstNI bslI
aciI
apyI[dcm+]
fnu4HI
hgaI
haeIII/palI
bsoFI cac8I
aciI
drdI
haeI nlaIV
haeIII/palI
nlaIV
sfaNI
taqI
mnlI
4701 AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC
TTCCGGTCCT TGGCATTTTT CCGGCGCAAC GACCGCAAAA AGGTATCCGA GGCGGGGGGA CTGCTCGTAG TGTTTTTAGC TGCGAGTTCA GTCTCCACCG

FIG. 41P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scrFI mvaI
mvaI ecoRII
ecoRII dsaV
dsaV bstNI
bstNI apyI[dcm+]
apyI[dcm+] bsaJI
bsaJI aluI
aluI mnlI
mnlI hhaI/cfoI
hhaI/cfoI hinPI
hinPI haeII
haeII aluI
aluI scfI
scfI ddeI
ddeI aluI
aluI alw44I/snoI
alw44I/snoI apaLI/snoI
apaLI/snoI bmyI
bmyI bsiHKAI
bsiHKAI bsp1286
bsp1286 hgiAI/aspHI
hgiAI/aspHI
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GAAAGAGGGA AGCCCTTCGC ACCGCGAAAG AGTATCGAGT GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT TCGACCCGAC ACACGTGCTT
fnu4HI fnu4HI
bsoFI bsoFI
nspBII nspBII
aciI aciI
hinPI hinPI
mspI mspI
maeIII maeIII
mcrI mcrI
bbvI bbvI
bsaWI bsaWI
bsiEI bsiEI
hhaI/cfoI hhaI/cfoI
hpaII hpaII
pleI pleI
hinfI hinfI
mspI mspI
hpaII hpaII
scrFI scrFI
nciI nciI
dsaV dsaV
cauII cauII
alwNI[dcm-] alwNI[dcm-]
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bsoFI bsoFI
fnu4HI fnu4HI
bsoFI bsoFI
bbvI bbvI
maeIII maeIII
bsrI bsrI
bbvI bbvI
bsrI bsrI
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GGGGGGCAAG TCGGGCTGGC GACGCGGAAT AGGCCATTGA TAGCAGAACT CAGGTTGGGC CATTCTGTGC TGAATAGCGG TGACCGTCGT CGGTGACCAT
bslI bslI
haeIII/palI haeIII/palI
rmaI rmaI
maeI maeI
hinPI hinPI
hhaI/cfoI hhaI/cfoI
mnlI mnlI
aciI aciI
scfI scfI
haeI haeI
bfaI bfaI
5101 ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC
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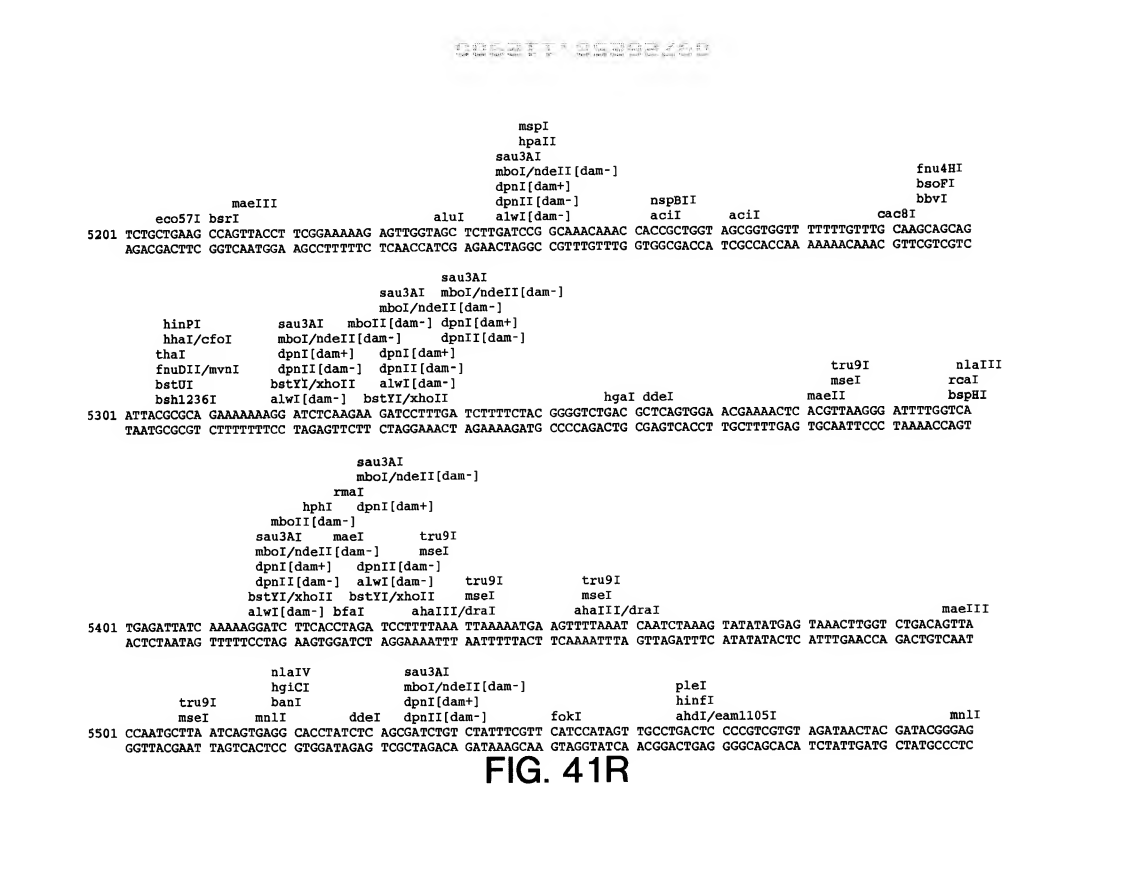
FIG. 41Q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nspBII
eco57I bsrI
aluI
alwI[dam-]
aciI
aciI
cac8I
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AGACGACTTC GGTCAATGGA AGCCTTTTTC TCAACCATCG AGAACTAGGC CGTTTGTTTG GTGGCGACCA TCGCCACCAA AAAAACAAAC GTTCGTCGTC
sau3AI
sau3AI mboI/ndeII[dam-]
mboI/ndeII[dam-]
hinPI
sau3AI
mboII[dam-]
dpnI[dam+]
hhaI/cfoI
mboI/ndeII[dam-]
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thaI
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dpnI[dam+]
fnuDII/mvnI
dpnII[dam-]
dpnII[dam-]
tru9I
nlaIII
bstUI
bstYI/xhoII
alwI[dam-]
mseI
rcaI
bsh1236I
alwI[dam-]
bstYI/xhoII
hgaI ddeI
maeII
bspHI
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sau3AI
mboI/ndeII[dam-]
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dpnI[dam+]
mboII[dam-]
sau3AI
maeI
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mboI/ndeII[dam-]
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dpnI[dam+]
dpnII[dam-]
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alwI[dam-]
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tru9I
bstYI/xhoII
bstYI/xhoII
mseI
mseI
alwI[dam-]
bfaI
ahaIII/draI
ahaIII/draI
maeIII
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sau3AI
hgiCI
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banI
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mseI
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ddeI
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fokI
ahdI/eam1105I
mnlI
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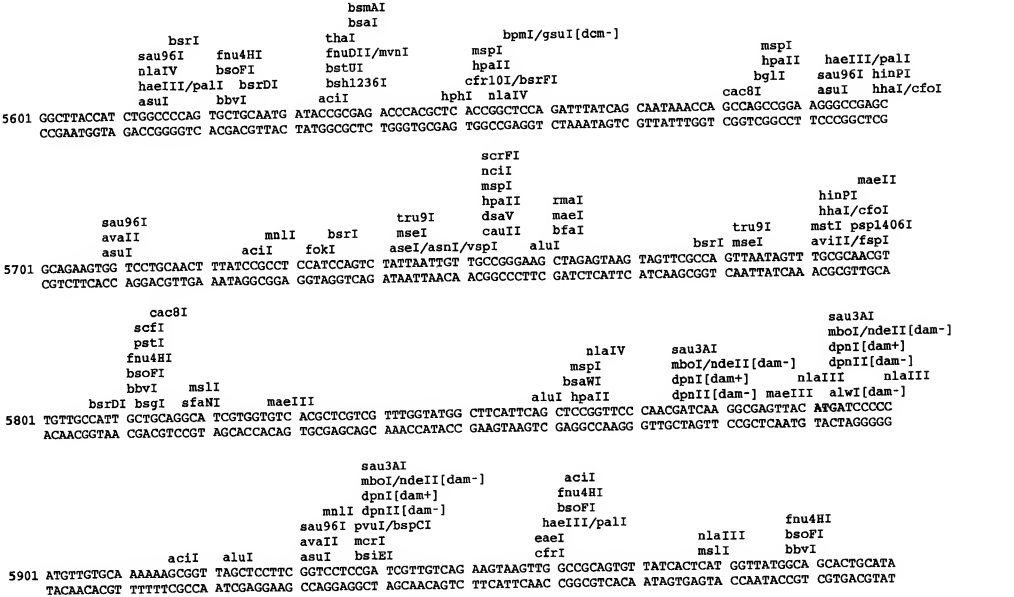
FIG. 41R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hpaII
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bsoFI
bstUI
cfr10I/bsrFI
bglI
sau96I hinPI
haeIII/palI
bsrDI
bsh1236I
hphI nlaIV
cac8I
asuI
hhaI/cfoI
asuI
bbvI
aciI
5601 GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC
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nciI
mspI
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hpaII
rmaI
hinPI
sau96I
dsaV
maeI
hhaI/cfoI
tru9I
avaII
mnlI
bsrI
mseI
cauII
bfaI
tru9I
mstI psp1406I
asuI
aciI
fokI
aseI/asnI/vspI
aluI
bsrI mseI
aviII/fspI
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CGTCTTCACC AGGACGTTGA AATAGGCGGA GGTAGGTCAG ATAATTAACA ACGGCCCTTC GATCTCATTC ATCAAGCGGT CAATTATCAA ACGCGTTGCA
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scfI
mboI/ndeII[dam-]
pstI
dpnI[dam+]
nlaIV
sau3AI
fnu4HI
mspI
mboI/ndeII[dam-]
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dpnI[dam+]
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bbvI
mslI
aluI hpaII
dpnII[dam-] maeIII
alwI[dam-]
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sfaNI
maeIII
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ACAACGGTAA CGACGTCCGT AGCACCACAG TGCGAGCAGC AAACCATACC GAAGTAAGTC GAGGCCAAGG GTTGCTAGTT CCGCTCAATG TACTAGGGGG
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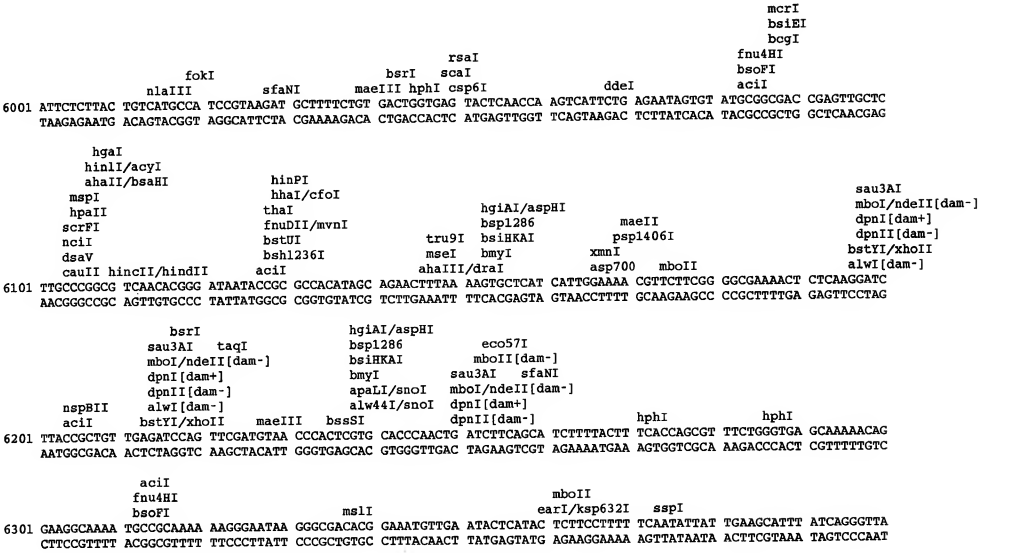
aciI
mboI/ndeII[dam-]
fnu4HI
dpnI[dam+]
bsoFI
mnlI dpnII[dam-]
haeIII/palI
fnu4HI
sau96I pvuI/bspCI
eaeI
bsoFI
avaII mcrI
nlaIII
cfrI
mslI
bbvI
aciI
aluI
asuI
bsiEI
5901 ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA
TACAACACGT TTTTTCGCCA ATCGAGGAAG CCAGGAGGCT AGCAACAGTC TTCATTCAAC CGGCGTCACA ATAGTGAGTA CCAATACCGT CGTGACGTAT

FIG. 41S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bsoFI
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rsaI
fokI
bsrI scaI
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6001 ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC
TAAGAGAATG ACAGTACGGT AGGCATTCTA CGAAAAGACA CTGACCACTC ATGAGTTGGT TCAGTAAGAC TCTTATCACA TACGCCGCTG GCTCAACGAG
hgaI
hinlI/acyI
ahaII/bsaHI
hinPI
hhaI/cfoI
sau3AI
mspI
mboI/ndeII[dam-]
hpaII
thaI
hgiAI/aspHI
bsp1286
maeII
dpnI[dam+]
scrFI
fnuDII/mvnI
psp1406I
dpnII[dam-]
nciI
bstUI
tru9I
bsiHKAI
bstYI/xhoII
dsaV
bsh1236I
mseI
bmyI
xmnI
alwI[dam-]
cauII hincII/hindII
aciI
ahaIII/draI
asp700
mboII
6101 TTGCCCGGCG TCAACACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT CTCAAGGATC
AACGGGCCGC AGTTGTGCCC TATTATGGCG CGGTGTATCG TCTTGAAATT TTCACGAGTA GTAACCTTTT GCAAGAAGCC CCGCTTTTGA GAGTTCCTAG
bsrI
hgiAI/aspHI
sau3AI taqI
bsp1286
eco57I
mboI/ndeII[dam-]
bsiHKAI
mboII[dam-]
dpnI[dam+]
bmyI
sau3AI sfaNI
dpnII[dam-]
apaLI/snoI
mboI/ndeII[dam-]
nspBII
alwI[dam-]
alw44I/snoI
dpnI[dam+]
aciI
bstYI/xhoII maeIII bssSI
dpnII[dam-]
hphI
hphI
6201 TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG
AATGGCGACA ACTCTAGGTC AAGCTACATT GGGTGAGCAC GTGGGTTGAC TAGAAGTCGT AGAAAATGAA AGTGGTCGCA AAGACCCACT CGTTTTTGTC
aciI
fnu4HI
bsoFI
mboII
msII
earI/ksp632I sspI
6301 GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA
CTTCCGTTTT ACGGCGTTTT TTCCCTTATT CCCGCTGTGC CTTTACAACT TATGAGTATG AGAAGGAAAA AGTTATAATA ACTTCGTAAA TAGTCCCAAT

FIG. 41T

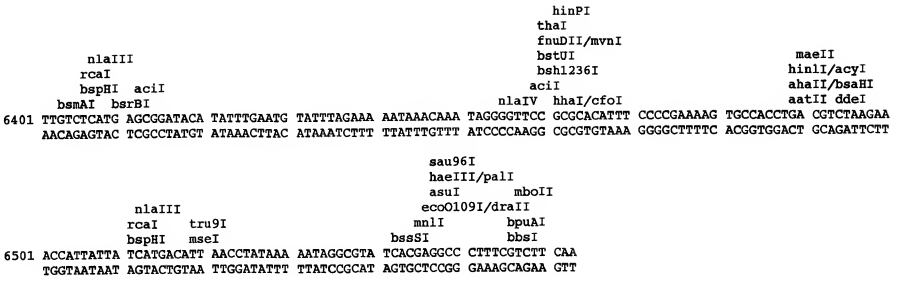

hinPI
thaI
fnuDII/mvnI
nlaIII
bstUI
maeII
rcaI
bsh1236I
hinlI/acyI
bspHI aciI
aciI
ahaII/bsaHI
bsmAI bsrBI
nlaIV hhaI/cfoI
aatII ddeI
6401 TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG TGCCACCTGA CGTCTAAGAA
AACAGAGTAC TCGCCTATGT ATAAACTTAC ATAAATCTTT TTATTTGTTT ATCCCCAAGG CGCGTGTAAA GGGGCTTTTC ACGGTGGACT GCAGATTCTT
sau96I
haeIII/palI
asuI mboII
nlaIII
eco0109I/draII
rcaI tru9I
mnlI bpuAI
bspHI mseI
bssSI bbsI
6501 ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTCTT CAA
TGGTAATAAT AGTACTGTAA TTGGATATTT TTATCCGCAT AGTGCTCCGG GAAAGCAGAA GTT

FIG. 41U

20040177-062502

```
>length: 6563

aatII(GACGTC):          1645 6489
acc65I(GGTACC):         403 823
accI(GTMKAC):           1093 1963 4449
accIII(TCCGGA):         3867[dam-]
aciI(CCGC):             178 542 805 877 1340 1750 1826 2011 2039 2043 2182 2242 2384 2492 2501 2504
                        2628 2781 2784 2787 2906 2926 3005 3045 3094 3141 3226 3241 3309 3342 3367 3412
                        3436 3448 3490 3544 3597 3613 3619 3700 3838 3967 3970 3981 4139 4155 4210 4266
                        4351 4390 4400 4442 4467 4505 4518 4544 4561 4604 4611 4632 4723 4751 4878 4897
                        5018 5128 5263 5272 5634 5725 5916 5962 6083 6127 6204 6313 6412 6459
acyI                    see hinlI
aflIII(ACRYGT):         1307 4678
ageI(ACCGGT):           1788
ahaII/bsaHI(GRCGYC):    1645 1813 2616 2637 2751 3408 6107 6489
ahaIII/draI(TTTAAA):    5435 5454 6146
ahdI/eam1105I(GACNNNNNGTC): 346 5566
aluI(AGCT):             72 121 252 320 398 532 589 648 1126 1144 1167 1325 1386 1906 2054 2075 2126
                        2218 2233 2889 3292 4202 4259 4270 4319 4338 4619 4845 4935 4981 5238 5759 5859
                        5922
alw44I/snoI(GTGCAC):    1831 4494 4992 6238
alwI[dam-](GGATC):      412 413 712 713 1171 1471 2578 2579 3300 3870 5245 5319 5331 5416 5429 5893
                        6196 6214
alwNI[dcm-](CAGNNNCTG): 1117 1385 5089
apaI(GGGCCC):           1695
apaLI/snoI(GTGCAC):     1831 4494 4992 6238
apoI(RAATTY):           1 391 4093
apyI[dcm+](CCWGG):      640 999 1347 1357 1449 1665 1713 1755 1764 2333 3262 3645 4705 4826 4839
aseI/asnI/vspI(ATTAAT): 5742
asnI                    see aseI
asp700(GAANNNNTTC):     905 930 4234 6166
asp718(GGTACC):         403 823
aspHI                   see hgiAI
aspI                    see tth111I
asuI(GGNCC):            1119 1195 1425 1434 1446 1512 1695 1696 1752 2155 2375 2727 3002 3090 3339 3463
```

FIG. 41V

Stop Template Primer

SL.97.2 5' CAT GGT ATA GGT TAA ACT TAT TTA CAC 3'

NNS Randomization Primer

SL.97.3 5' CAT GGT ATA GGT NNS ACT TAT TTA CAC 3'

FIG. 42

Randomization of Position N35 of Variable Light Chain CDR-1
Amino Acid Frequency

Phage Display (NNS Codon Library) Sort #3

Amino Acid	Frequency	% Total	IC50 (nM)
Asparagine (wt)	1	5.6	4.9
Glycine	6	16.6	3.1
Aspartic Acid	3	16.6	3.1
Glutamic Acid	4	22.2	0.1
Alanine	2	5.6	0.2
Lysine	1	5.6	ND
Serine	1	1.9	ND

FIG. 43A

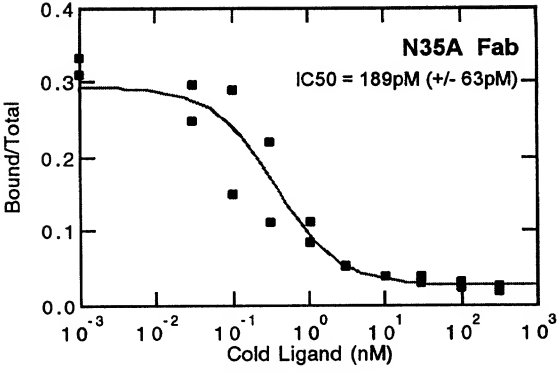


FIG. 43B

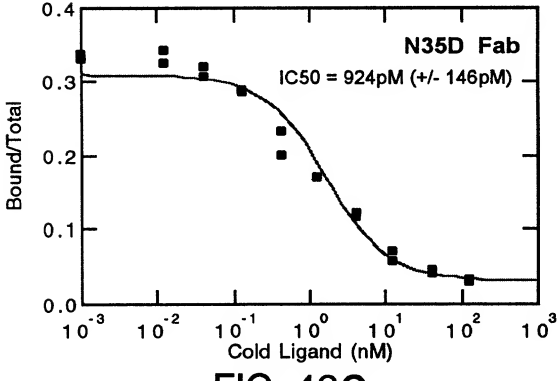


FIG. 43C

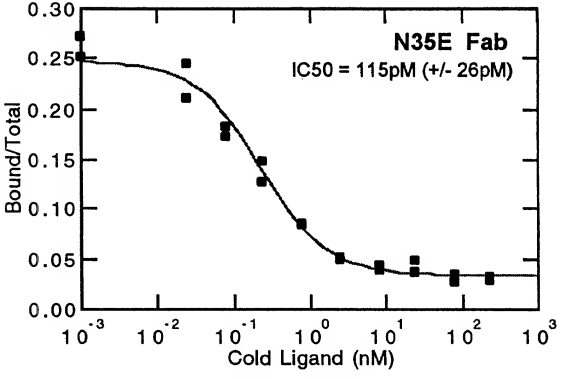


FIG. 43D

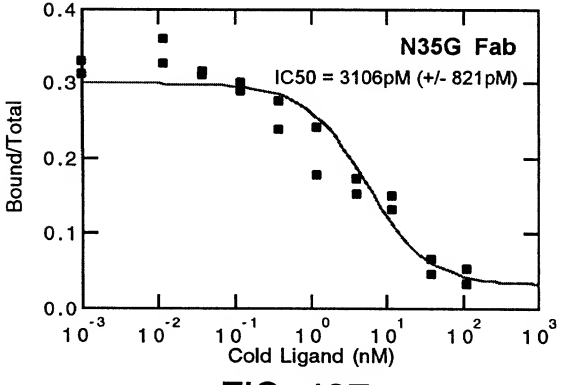
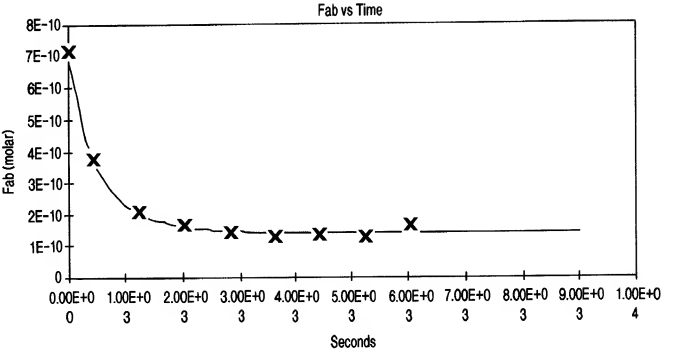


FIG. 43E



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.F(ab')2.

SAMPLE	ka	kd	Kd
6G4V11N35A-Fab	ND	ND	114pM
6G4V11N35A-F(ab')$_2$	2.0×10^6	2.1×10^{-4}	109pM
6G4V11N35E-Fab	4.7×10^6	2.6×10^{-4}	54pM

FIG. 44

```
  1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
    TACTTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
-23 M  K  K  N     I  A  F     L  L  A     S  M  F  V     F  S  I     A  T  N

 61 GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC TGTCCGCCTC TGTGGGCGAT
    CGTATGCGAC TATAGGTCTA CTGGGTCAGG GGCTCGAGGG ACAGGCGGAG ACACCCGCTA
 -3 A  Y  A  D     I  Q  M     T  Q  S     P  S  S  L     S  A  S     V  G  D

121 AGGGTCACCA TCACCTGCAG GTCAAGTCAA AGCTTAGTAC ATGGTATAGG TGAGACGTAT
    TCCCAGTGGT AGTGGACGTC CAGTTCAGTT TCGAATCATG TACCATATCC ACTCTGCATA
 18 R  V  T  I     T  C  R  S  S  Q  S  L  V  H  G  I  G  E  T  Y

181 TTACACTGGT ATCAACAGAA ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC
    AATGTGACCA TAGTTGTCTT TGGTCCTTTT CGAGGCTTTG ATGACTAAAT GTTTCATAGG
 38 L  H  W  Y     Q  Q  K     P  G  K     A  P  K  L     L  I  Y     K  V  S

241 AATCGATTCT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT
    TTAGCTAAGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG CCTAAAGTGA
 58 N  R  F  S     G  V  P     S  R  F     S  G  S  G     S  G  T     D  F  T

301 CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC ACAGAGTACT
    GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAAG TGTCTCATGA
 78 L  T  I  S     S  L  Q     P  E  D     F  A  T  Y     Y  C  S  Q  S  T

361 CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA TCAAACGAAC TGTGGCTGCA
    GTACAGGGCG AGTGCAAACC TGTCCCATGG TTCCACCTCT AGTTTGCTTG ACACCGACGT
 98 H  V  P  L  T     F  G     Q  G  T     K  V  E  I     K  R  T     V  A  A

421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT
    GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA
118 P  S  V  F     I  F  P     P  S  D     E  Q  L  K     S  G  T     A  S  V

481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
    CACACGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
138 V  C  L  L     N  N  F     Y  P  R     E  A  K  V     Q  W  K     V  D  N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
    CGGGAGGTTA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT CCTGTCGTGG
158 A  L  Q  S     G  N  S     Q  E  S     V  T  E  Q     D  S  K     D  S  T

601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
    ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG
178 Y  S  L  S     S  T  L     T  L  S     K  A  D  Y     E  K  H     K  V  Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
    CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT
198 A  C  E  V     T  H  Q     G  L  S     S  P  V  T     K  S  F     N  R  G

721 GAGTGTTAAG CTGATCCTCT ACGCCGGACG CATCGTGGCC CTAGTACGCA ACTAGTCGTA
    CTCACAATTC GACTAGGAGA TGCGGCCTGC GTAGCACCGG GATCATGCGT TGATCAGCAT
218 E  C  O
```

FIG. 45

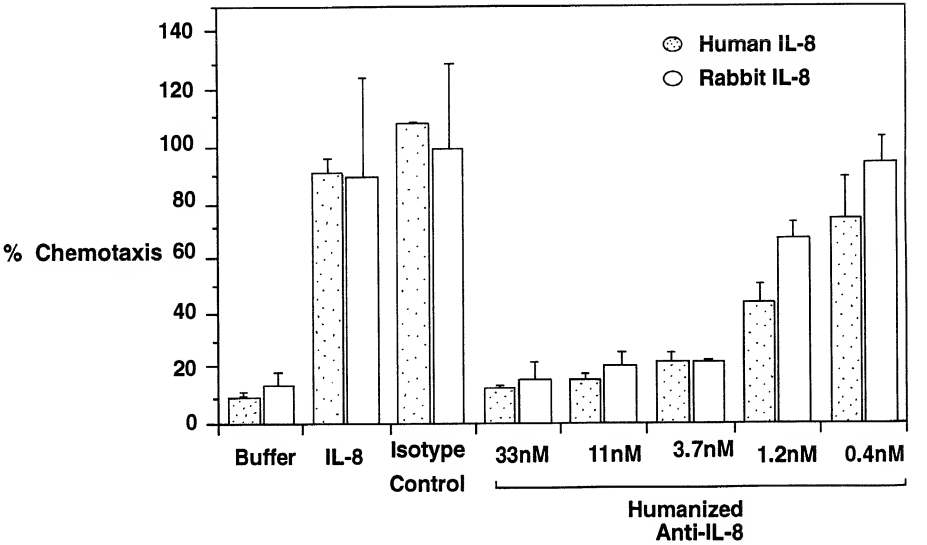

140
120
100
80
% Chemotaxis 60
40
20
0
Human IL-8
Rabbit IL-8
Buffer
IL-8
Isotype Control
33nM
11nM
3.7nM
1.2nM
0.4nM
Humanized Anti-IL-8

FIG. 46

N35AH1upr

5'-CT**A**GTGCAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCCTGTGCAGCTTCTGGCTACTCCTTC-3'

N35AH1lwr

5'-TCGAGAAGGAGTAGCCAGAAGCTGCACAGGACAAACGGAGTGAGCCCCCTGGCTGCACCAGGCCACCGCCAGACTGCACT

AG-3'

Bold indicates nucleotide change destroying PvuII site.

FIG. 47

```
> Wed May  7 18:27:36 1997
> /home/ruby/vc/Immbio/afan/ss.p6G425v11.N35A.choSD
> sites: std
> length: 8120 (circular)
>This has the pSVI backbone with the pRK7 cloning linker (pSVI7) and the intron DHFR(ID)
>made from pSVI.WTSD.D by adding a linearization linker(LL) into the HpaI site

                cac8I
               aluI
              sstI
              sacI                          sau3AI  aluI                                  scrFI
              hgiJII                        mboI/ndeII[dam-]                              mvaI
              hgiAI/aspHI                   dpnI[dam+]                                    ecoRII
              ecl136II                     pvuI/bspCI                                     dsaV
              bsp1286                   pleI dpnII[dam-]                                  bstNI
              bsiHKAI                   hinfI  taqI[dam-]                                 apyI[dcm+]
              bmyI                  rmaI    mcrI    pvuII                                 bsaJI
              banII                 maeI    bsiEI   nspBII                       bsmFI   nlaIV     cac8I
             taqI                   bfaI    taqI[dam-]                                  |       |
    1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
      AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                    sfaNI                                   scrFI                   sfaNI
                   ppuI0I                  scrFI        mvaI                       ppuI0I
                   nsiI/avaIII             mvaI                 ecoRII            nsiI/avaIII
                  nlaIII                   ecoRII       dsaV                  nlaIII
                 sphI                      dsaV         bstNI                      sphI
                 nspI                      bstNI                 apyI[dcm+]            nspI
                 nspHI                     apyI[dcm+]         bsaJI                    nspHI
                 cac8I                     sexAI           bsmFI   nlaIV     cac8I      cac8I
  101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
      CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                  nlaIII
                                                                                 styI
                                                                                 ncoI
                                                                              bslI dsaI
                    aciI                    aciI                              aciI bsaJI
                  bsmFI           aciI  fokI              aciI bsrI  aciI
  201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
      CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA
```

FIG. 48A

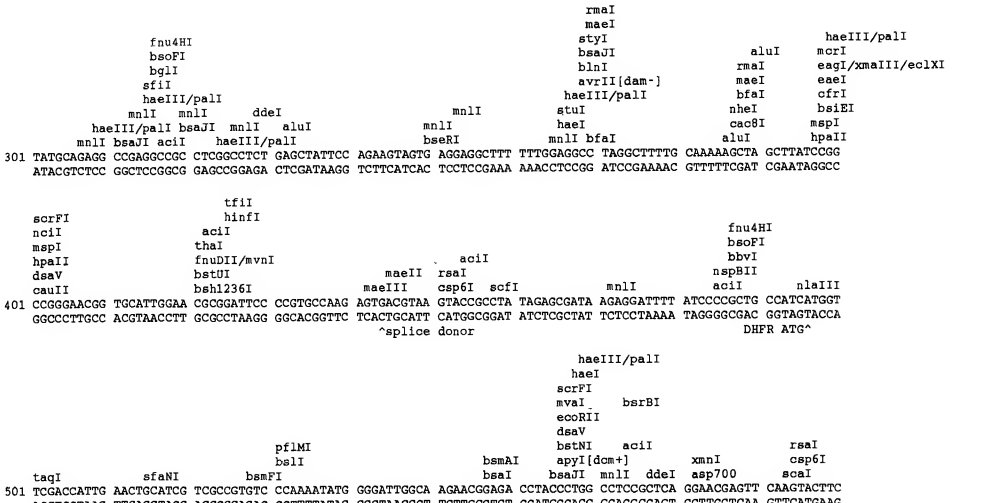

rmaI
maeI
styI
bsaJI
blnI
avrII[dam-]
haeIII/palI
stuI
haeI
mnlI bfaI
aluI
fnu4HI
bsoFI
bglI
sfiI
haeIII/palI
mnlI mnlI ddeI
haeIII/palI bsaJI mnlI aluI
mnlI bsaJI acil haeIII/palI
mnlI
bseRI
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
bsiEI
mspI
hpaII
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
tfiI
hinfI
scrFI
nciI
aciI
mspI
thaI
hpaII
fnuDII/mvnI
aciI
dsaV
bstUI
maeII rsaI
cauII
bsh1236I
maeIII csp6I scfI
mnlI
aciI
nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA
^splice donor
fnu4HI
bsoFI
bbvI
nspBII
DHFR ATG^
haeIII/palI
haeI
scrFI
mvaI bsrBI
ecoRII
dsaV
bstNI aciI
rsaI
pflMI
bslI
bsmAI apyI[dcm+]
xmnI
csp6I
taqI sfaNI bsmFI
bsaI bsaJI mnlI ddeI asp700
scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

FIG. 48B

scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
sexAI
tfiI
hinfI
ddeI mboII taqI
tru9I
mseI
ahaIII/draI
eco57I
mboII
earI/ksp632I
mnlI
tfiI
hinfI hphI
alwNI[dcm-]
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
bmyI
mnlI aluI
bssSI banII
bslI bseRI
bstXI fokI sfaNI mseI
tru9I
mseI
aseI/asnI/vspI
ddeI
tru9I
aflII/bfrI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
naeIII/palI
haeI
scrFI
mvaI
ecoRII
dsaV tfiI
bstNI nlaIII
apyI[dcm+] hinfI
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
ddeI pleI
hinfI
mspI
hpaII
bsaWI
accI nlaIII
mnlI
801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

FIG. 48C

hgaI
hinlI/acyI
ahaII/bsaHI
scrFI
mvaI
mnlI
ecoRII
dsaV
bstNI
ecoNI
apyI[dcm+]
mnlI
bsaJI
bslI ddeI
nlaIII
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
maeII
dpnII[dam-]
aflIII
maeIII alwI[dam-]
apoI
maeIII
mnlI
901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
sau96I
avaII
sfaNI
asuI
mnlI
sfaNI
accI
mboII
mboII
mnlI
aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
^END DHFR
styI
bsaJI
sau3AI
mboI/ndeII[dam-]
nlaIII
styI
dpnI[dam+]
aluI
ncoI
dpnII[dam-]
fnu4HI
tru9I
ppu10I
dsaI bsmFI
alwI[dam-]
bsoFI
mseI
nsiI/avaIII
bsaJI
cac8I
bstYI/xhoII
bbvI
aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
sau96I
avaII
asuI
scrFI
mvaI
ecoRII

FIG. 48D

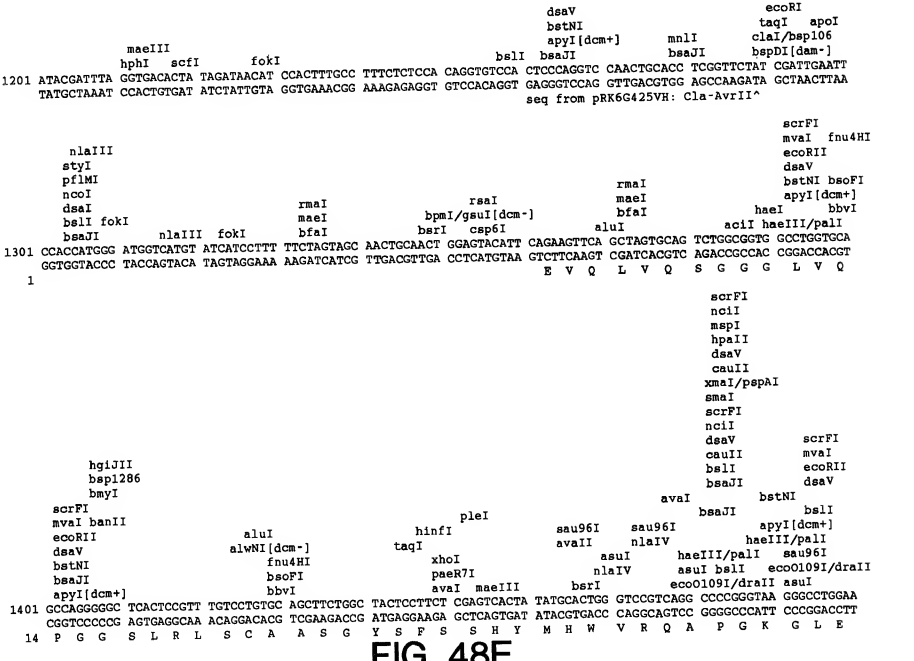

dsaV
bstNI
apyI[dcm+]
mnlI
bsaJI
ecoRI
taqI apoI
claI/bsp106
bspDI[dam-]
maeIII
hphI scfI fokI
bslI bsaJI
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA
seq from pRK6G425VH: Cla-AvrII^
scrFI
mvaI fnu4HI
ecoRII
dsaV
bstNI bsoFI
apyI[dcm+]
haeI bbvI
sciI haeIII/palI
nlaIII
styI
pflMI
ncoI
dsaI
bslI fokI
bsaJI
nlaIII fokI
rmaI
maeI
bfaI
bsrI
rsaI
bpmI/gsuI[dcm-]
csp6I
rmaI
maeI
bfaI
aluI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTAGTGCAG TCTGGCGGTG GCCTGGTGCA
GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGATCACGTC AGACCGCCAC CGGACCACGT
1 E V Q L V Q S G G G L V Q
scrFI
nciI
mspI
hpaII
dsaV
cauII
xmaI/pspAI
smaI
scrFI
nciI
dsaV
cauII
bslI
bsaJI
scrFI
mvaI
ecoRII
dsaV
hgiJII
bsp1286
bmyI
scrFI
mvaI banII
ecoRII
dsaV
bstNI
bsaJI
apyI[dcm+]
aluI
alwNI[dcm-]
fnu4HI
bsoFI
bbvI
pleI
hinfI
taqI
xhoI
paeR7I
aviI maeIII
sau96I
aviII
asuI
nlaIV
bsrI
avaI
bsaJI
bstNI
sau96I
nlaIV
asuI haeIII/palI
eco0109I/draII asuI
bslI
apyI[dcm+]
sau96I
haeIII/palI
bslI
asuI bslI eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGCTTCTGGC TACTCCTTCT CGAGTCACTA TATGCACTGG GTCCGTCAGG CCCCGGGTAA GGGCCTGGAA
CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCGAAGACCG ATGAGGAAGA GCTCAGTGAT ATACGTGACC CAGGCAGTCC GGGGCCCATT CCCGGACCTT
14 P G G S L R L S C A A S G Y S F S S H Y M H W V R Q A P G K G L E

FIG. 48E

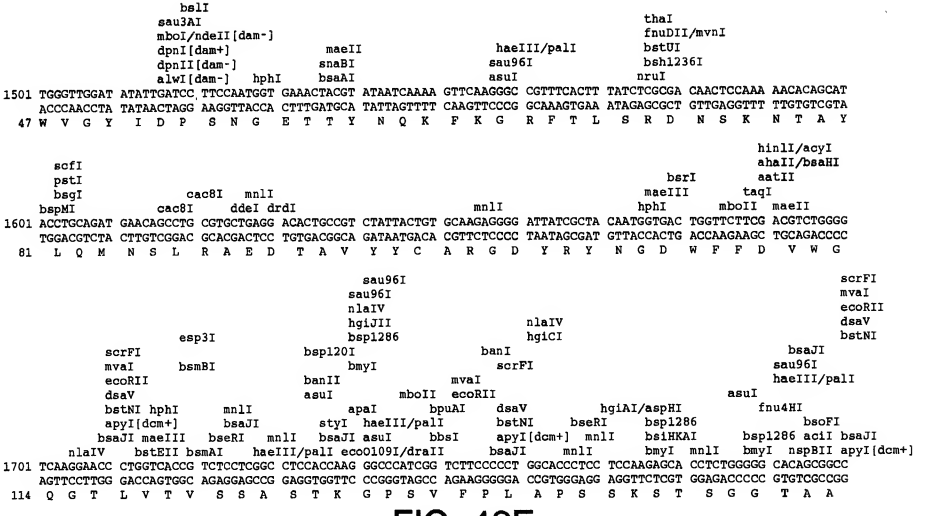
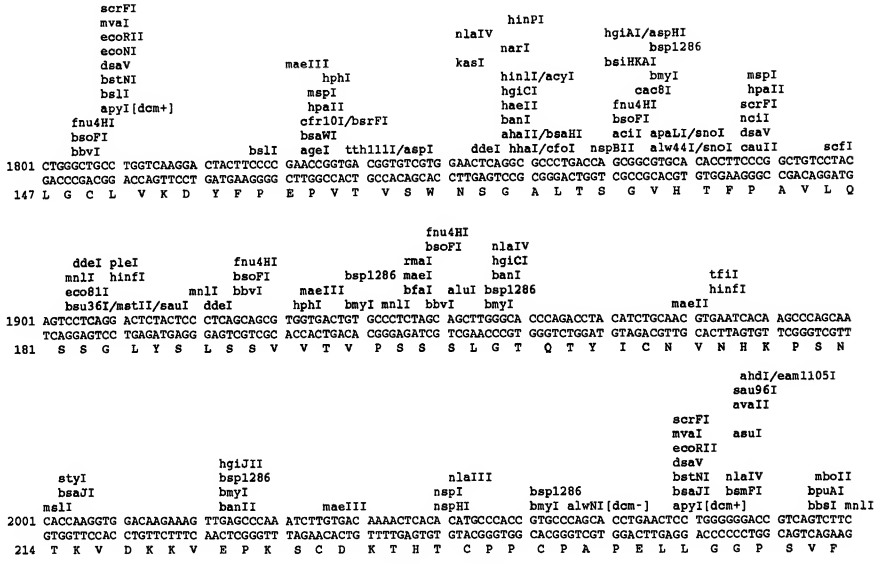

bslI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
hphI
maeII
snaBI
bsaAI
haeIII/palI
sau96I
asuI
thaI
fnuDII/mvnI
bstUI
bsh1236I
nruI
1501 TGGGTTGGAT ATATTGATCC TTCCAATGGT GAAACTACGT ATAATCAAAA GTTCAAGGGC CGTTTCACTT TATCTCGGGA CAACTCCAAA AACACAGCAT
ACCCAACCTA TATAACTAGG AAGGTTACCA CTTTGATGCA TATTAGTTTT CAAGTTCCCG GCAAAGTGAA ATAGAGCGCT GTTGAGGTTT TTGTGTCGTA
47 W V G Y I D P S N G E T T Y N Q K F K G R F T L S R D N S K N T A Y
hinlI/acyI
ahaII/bsaHI
aatII
taqI
scfI
pstI
bsgI
cac8I
mnlI
bsrI
maeIII
bspMI
cac8I
ddeI
drdI
mnlI
hphI
mboII
maeII
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTACTGT GCAAGAGGGG ATTATCGCTA CAATGGTGAC TGGTTCTTCG ACGTCTGGGG
TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATGACA CGTTCTCCCC TAATAGCGAT GTTACCACTG ACCAAGAAGC TGCAGACCCC
81 L Q M N S L R A E D T A V Y Y C A R G D Y R Y N G D W F F D V W G
scrFI
mvaI
ecoRII
dsaV
bstNI
sau96I
sau96I
nlaIV
hgiJII
bsp1286
esp3I
scrFI
mvaI
bsmBI
ecoRII
dsaV
bstNI hphI
mnlI
apyI[dcm+]
bsaJI
bsp120I
banI
bmyI
scrFI
banII
mvaI
asuI
mboII ecoRII
apaI
bpuAI
dsaV
styI haeIII/palI
nlaIV
hgiCI
hgiAI/aspHI
bstNI
bseRI
bsp1286
bsaJI maeIII
bseRI
mnlI
bsaJI asuI
bbsI
apyI[dcm+]
mnlI
bsiHKAI
bsaJI
sau96I
haeIII/palI
asuI
fnu4HI
bsp1286 aciI bsaJI
bsoFI
nlaIV
bstEII bsmAI
haeIII/palI eco0109I/draII
bsaJI
mnlI
bmyI mnlI
bmyI nspBII apyI[dcm+]
1701 TCAAGGAACC CTGGTCACCG TCTCCTCGGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA CCTCTGGGGG CACAGCGGCC
AGTTCCTTGG GACCAGTGGC AGAGGAGCCG GAGGTGGTTC CCGGGTAGCC AGAAGGGGGA CCGTGGGAGG AGGTTCTCGT GGAGACCCCC GTGTCGCCGG
114 Q G T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A

FIG. 48F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mvaI
ecoRII
econI
dsaV
bstNI
bslI
apyI[dcm+]
fnu4HI
bsoFI
bbvI
maeIII
hphI
mspI
hpaII
cfr10I/bsrFI
bsaWI
bslI ageI tth111I/aspI
hinPI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII
banI
ahaII/bsaHI
ddeI hhaI/cfoI nspBII
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
cac8I
fnu4HI
bsoFI
acil apaLI/snoI
mspI
hpaII
scrFI
nciI
dsaV
scfI
1801 CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTGCA CACCTTCCCG GCTGTCCTAC
GACCCGACGG ACCAGTTCCT GATGAAGGGG CTTGGCCACT GCCACAGCAC CTTGAGTCCG CGGGACTGGT CGCCGCACGT GTGGAAGGGC CGACAGGATG
147 L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q
fnu4HI
bsoFI nlaIV
rmaI hgiCI
ddeI pleI fnu4HI
mnlI hinfI bsoFI bsp1286 maeI banI tfiI
eco81I mnlI bbvI maeIII bfaI aluI bsp1286 hinfI
bsu36I/mstII/sauI ddeI hphI bmyI mnlI bbvI bmyI maeII
1901 AGTCCTCAGG ACTCTACTCC CTCAGCAGCG TGGTGACTGT GCCCTCTAGC AGCTTGGGCA CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA
TCAGGAGTCC TGAGATGAGG GAGTCGTCGC ACCACTGACA CGGGAGATCG TCGAACCCGT GGGTCTGGAT GTAGACGTTG CACTTAGTGT TCGGGTCGTT
181 S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N
ahdI/eam1105I
sau96I
avaII
scrFI
mvaI asuI
ecoRII
dsaV
hgiJII
styI bsp1286 nlaIII bstNI nlaIV mboII
bsaJI bmyI nspI bsp1286 bsaJI bsmFI bpuAI
mslI banII maeIII nspHI bmyI alwNI[dcm-] apyI[dcm+] bbsI mnlI
2001 CACCAAGGTG GACAAGAAAG TTGAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC
GTGGTTCCAC CTGTTCTTTC AACTCGGGTT TAGAACACTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT GGACTTGAGG ACCCCCCTGG CAGTCAGAAG
214 T K V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F

FIG. 48G

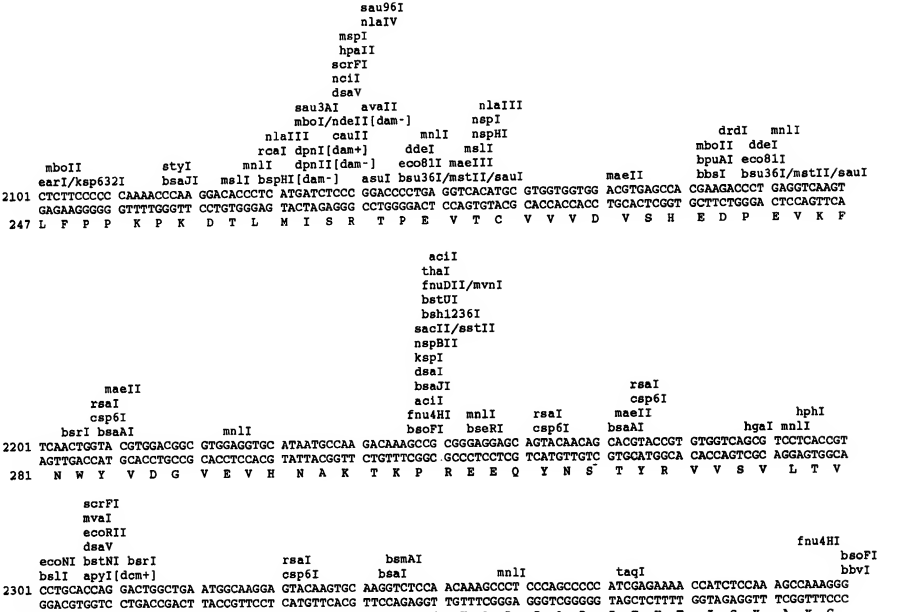

sau96I
nlaIV
mspI
hpaII
scrFI
nciI
dsaV
sau3AI avaII nlaIII
mboI/ndeII[dam-] nspI
nlaIII cauII mnlI nspHI drdI mnlI
rcaI dpnI[dam+] ddeI mslI mboII ddeI
mboII styI mnlI dpnI[dam-] eco81I maeIII bpuAI eco81I
earI/ksp632I bsaJI mslI bspHI[dam-] asuI bsu36I/mstII/sauI maeII bbsI bsu36I/mstII/sauI
2101 CTCTTCCCCC CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT
GAGAAGGGGG GTTTTGGGTT CCTGTGGGAG TACTAGAGGG CCTGGGGACT CCAGTGTACG CACCACCACC TGCACTCGGT GCTTCTGGGA CTCCAGTTCA
247 L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F
aciI
thaI
fnuDII/mvnI
bstUI
bsh1236I
sacII/sstII
nspBII
kspI
dsaI
maeII bsaJI rsaI
rsaI aciI csp6I hphI
csp6I fnu4HI mnlI rsaI maeII
bsrI bsaAI mnlI bsoFI bseRI csp6I bsaAI hgaI mnlI
2201 TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT
AGTTGACCAT GCACCTGCCG CACCTCCACG TATTACGGTT CTGTTTCGGC GCCCTCCTCG TCATGTTGTC GTGCATGGCA CACCAGTCGC AGGAGTGGCA
281 N W Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V
scrFI
mvaI
ecoRII
dsaV fnu4HI
ecoNI bstNI bsrI rsaI bsmAI bsoFI
bslI apyI[dcm+] csp6I bsaI mnlI taqI bbvI
2301 CCTGCACCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG
GGACGTGGTC CTGACCGACT TACCGTTCCT CATGTTCACG TTCCAGAGGT TGTTTCGGGA GGGTCGGGGG TAGCTCTTTT GGTAGAGGTT TCGGTTTCCC
314 L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G

FIG. 48H

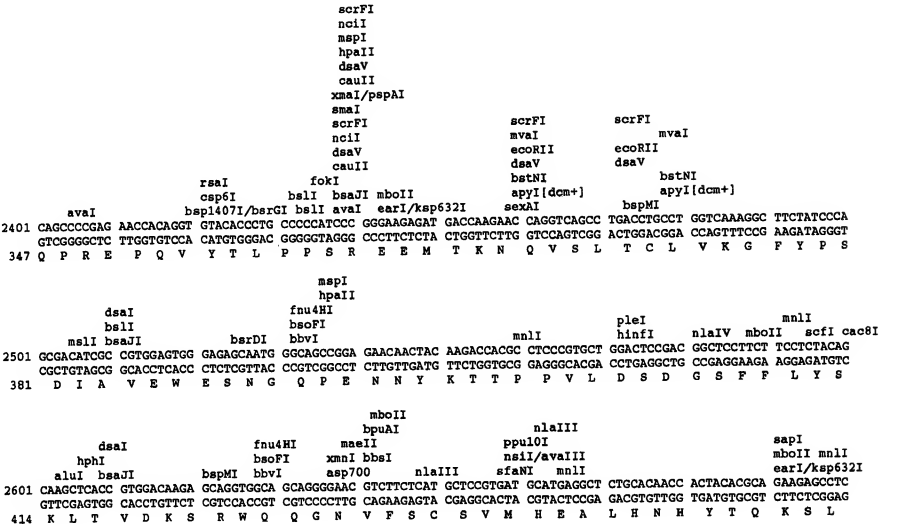
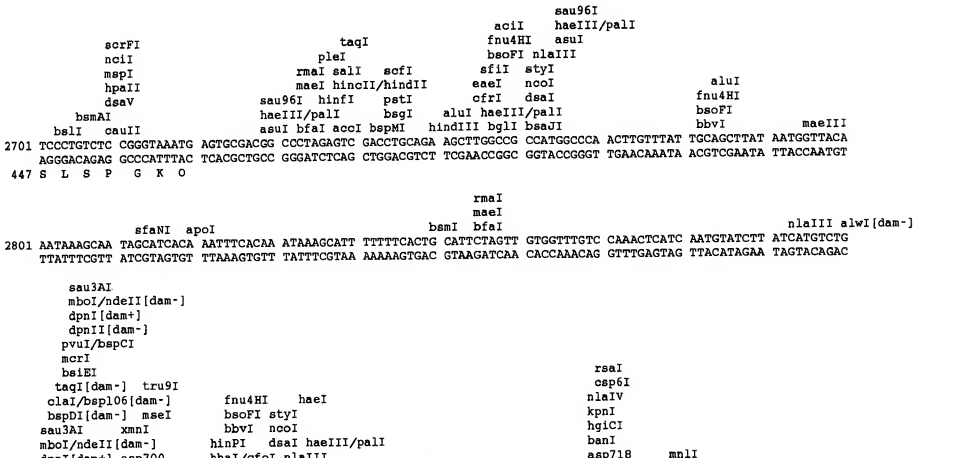

scrFI
nciI
mspI
hpaII
dsaV
cauII
xmaI/pspAI
smaI
scrFI
nciI
dsaV
cauII
rsaI
csp6I
fokI
bslI
bsaJI
mboII
scrFI
mvaI
ecoRII
dsaV
apyI[dcm+]
sexAI
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
bspMI
avaI
bsp1407I/bsrGI
bslI
avaI
earI/ksp632I
2401 CAGCCCCGAG AACCACAGGT GTACACCCTG CCCCCATCCC GGGAAGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA
GTCGGGGCTC TTGGTGTCCA CATGTGGGAC GGGGGTAGGG CCCTTCTCTA CTGGTTCTTG GTCCAGTCGG ACTGGACGGA CCAGTTTCCG AAGATAGGGT
347 Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S
mspI
hpaII
dsaI
bslI
mslI
bsaJI
fnu4HI
bsoFI
bsrDI
bbvI
mnlI
pleI
hinfI
mnlI
nlaIV
mboII
scfI
cac8I
2501 GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG
CGCTGTAGCG GCACCTCACC CTCTCGTTAC CCGTCGGCCT CTTGTTGATG TTCTGGTGCG GAGGGCACGA CCTGAGGCTG CCGAGGAAGA AGGAGATGTC
381 D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S
mboII
bpuAI
nlaIII
dsaI
hphI
aluI
bsaJI
fnu4HI
bsoFI
bspMI
bbvI
maeII
xmnI
bbsI
asp700
nlaIII
ppu10I
nsiI/avaIII
sfaNI
mnlI
sapI
mboII
mnlI
earI/ksp632I
2601 CAAGCTCACC GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC
GTTCGAGTGG CACCTGTTCT CGTCCACCGT CGTCCCCTTG CAGAAGAGTA CGAGGCACTA CGTACTCCGA GACGTGTTGG TGATGTGCGT CTTCTCGGAG
414 K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L

FIG. 48I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acil haeIII/palI
fnu4HI asuI
bsoFI nlaIII
sfiI styI
eaeI ncoI
cfrI dsaI
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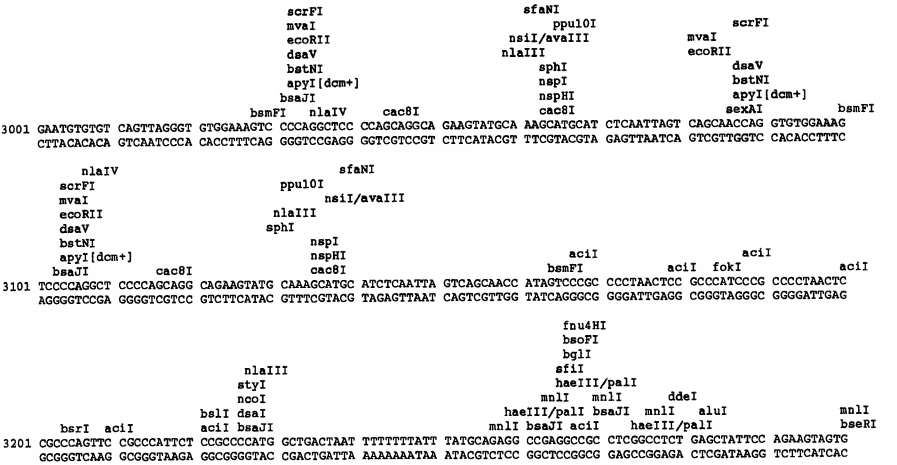
mspI
hpaII
dsaV
bsmAI
taqI
pleI
rmaI salI scfI
maeI hincII/hindII
sau96I hinfI pstI
haeIII/palI bsgI aluI haeIII/palI
asuI bfaI accI bspMI hindIII bglI bsaJI
aluI
fnu4HI
bsoFI
bbvI maeIII
bslI cauII
2701 TCCCTGTCTC CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTGGCCG CCATGGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA
AGGGACAGAG GCCCATTTAC TCACGCTGCC GGGATCTCAG CTGGACGTCT TCGAACCGGC GGTACCGGGT TGAACAAATA ACGTCGAATA TTACCAATGT
447 S L S P G K O
rmaI
maeI
sfaNI apoI
bsmI bfaI
nlaIII alwI[dam-]
2801 AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG
TTATTTCGTT ATCGTAGTGT TTAAAGTGTT TATTTCGTAA AAAAAGTGAC GTAAGATCAA CACCAAACAG GTTTGAGTAG TTACATAGAA TAGTACAGAC
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
pvuI/bspCI
mcrI
bsiEI
taqI[dam-] tru9I
claI/bspl06[dam-]
bspDI[dam-] mseI
sau3AI xmnI
mboI/ndeII[dam-]
dpnI[dam+] asp700
dpnII[dam-] aseI/asnI/vspI
fnu4HI haeI
bsoFI styI
bbvI ncoI
hinPI dsaI haeIII/palI
hhaI/cfoI nlaIII
bsaJI
mnlI
mnlI
rsaI
csp6I
nlaIV
kpnI
hgiCI
banI
asp718 mnlI
acc65I ddeI aciI
2901 GATCGATCGG GAATTAATTC GGCGCAGCAC CATGGCCTGA AATAACCTCT GAAAGAGGAA CTTGGTTAGG TACCTTCTGA GGCGGAAAGA ACCATCTGTG
CTAGCTAGCC CTTAATTAAG CCGCGTCGTG GTACCGGACT TTATTGGAGA CTTTCTCCTT GAACCAATCC ATGGAAGACT CCGCCTTTCT TGGTAGACAC

FIG. 48J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mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
bsaJI
bsmFI nlaIV cac8I
sfaNI
ppu10I
nsiI/avaIII
nlaIII
sphI
nspI
nspHI
cac8I
acil
bsmFI
aciI fokI
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
sexAI
bsmFI
3001 GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG
CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC
nlaIV
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
bsaJI cac8I
sfaNI
ppu10I
nsiI/avaIII
nlaIII
sphI
nspI
nspHI
cac8I
acil
bsmFI
aciI fokI
aciI
3101 TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC
AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG
fnu4HI
bsoFI
bglI
sfiI
haeIII/palI
mnlI mnlI ddeI
haeIII/palI bsaJI mnlI aluI
mnlI bsaJI aciI haeIII/palI
nlaIII
styI
ncoI
bslI dsaI
bsrI aciI
aciI bsaJI
bseRI
3201 CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG
GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC

FIG. 48K

scrFI
nciI
mspI
rmaI
hpaII
maeI
dsaV
styI
haeIII/palI
bsaJI
aluI
mcrI
tfiI
blnI
rmaI
eagI/xmaIII/eclXI
hinfI
avrII[dam-]
maeI
eaeI
aciI
haeIII/palI
bfaI
cfrI
thaI
stuI
nheI
bsiEI
fnuDII/mvnI
haeI
cac8I
mspI cauII
bstUI
pleI
mnlI
mnlI bfaI
aluI
hpaII
bsh1236I
hinfI
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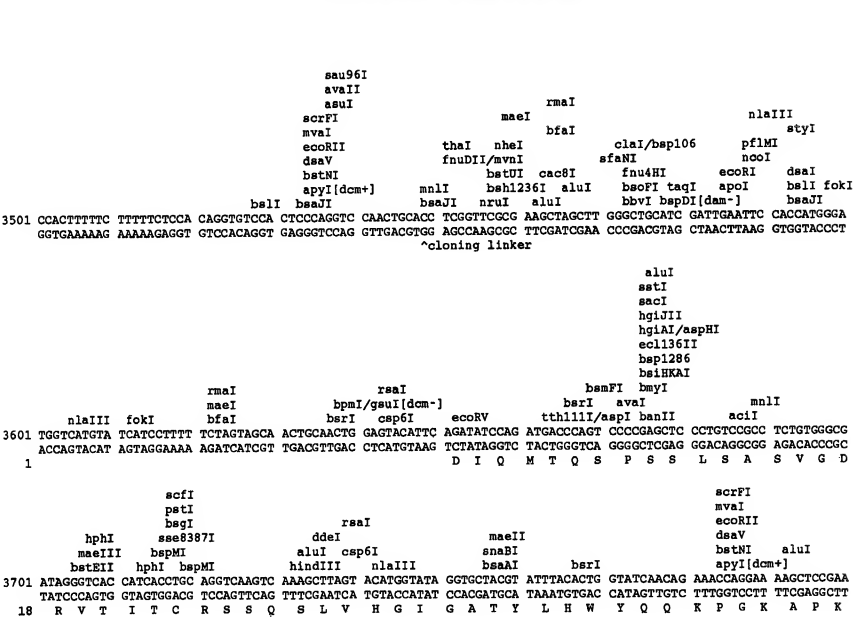
TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCAGTCCATT
^seq from pSV16B5-6G4VL: AvrII - HindIII frag
U1 matched splice donar^
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
taqI[dam-]
claI/bsp106[dam-]
fnu4HI
bspDI[dam-]
bsoFI
aciI
sau3AI
bstXI
thaI
mboI/ndeII[dam-]
aciI
scfI
sau96I
styI
fnuDII/mvnI tru9I
dpnI[dam+]
bstUI
mseI
dpnII[dam-]
rsaI
pleI
haeIII/palI
csp6I
scfI
hinfI
asuI
bsaJI
bsh1236I
aseI/asnI/vspI
alwI[dam-]
fokI
3401 GTACCGCCTA TAGAGTCTAT AGGCCCACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTTTGGATC GATCCTACTG ACACTGACAT
CATGGCGGAT ATCTCAGATA TCCGGGTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAAAACCTAG CTAGGATGAC TGTGACTGTA
^sp6 promoter
^removed ATG
^U2 match
lariat consensus^
IgG vH natural lariat restored^

FIG. 48L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avaII
asuI
rmaI
scrFI
maeI
nlaIII
mvaI
bfaI
styI
ecoRII
thaI nheI
claI/bsp106
pflMI
dsaV
fnuDII/mvnI
sfaNI
ncoI
bstNI
bstUI cac8I
fnu4HI
ecoRI dsaI
apyI[dcm+]
mnlI
bsh1236I aluI
bsoFI taqI
apoI
bslI fokI
bslI bsaJI
bsaJI nruI aluI
bbvI bspDI[dam-]
bsaJI
3501 CCACTTTTTC TTTTTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCGCG AAGCTAGCTT GGGCTGCATC GATTGAATTC CACCATGGGA
GGTGAAAAAG AAAAAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGCGC TTCGATCGAA CCCGACGTAG CTAACTTAAG GTGGTACCCT
^cloning linker
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
rmaI
rsaI
bsmFI bmyI
maeI
bpmI/gsuI[dcm-]
bsrI avaI
mnlI
nlaIII fokI
bfaI
bsrI csp6I
ecoRV
tth111I/aspI banII
aciI
3601 TGGTCATGTA TCATCCTTTT TCTAGTAGCA ACTGCAACTG GAGTACATTC AGATATCCAG ATGACCCAGT CCCCGAGCTC CCTGTCCGCC TCTGTGGGCG
ACCAGTACAT AGTAGGAAAA AGATCATCGT TGACGTTGAC CTCATGTAAG TCTATAGGTC TACTGGGTCA GGGGCTCGAG GGACAGGCGG AGACACCCGC
1 D I Q M T Q S P S S L S A S V G D
scfI
pstI
bsgI
scrFI
mvaI
rsaI
ecoRII
hphI
sse8387I
ddeI
maeII
dsaV
maeIII
bspMI
aluI csp6I
snaBI
bstNI aluI
bstEII hphI bspMI
hindIII nlaIII
bsaAI
bsrI
apyI[dcm+]
3701 ATAGGGTCAC CATCACCTGC AGGTCAAGTC AAAGCTTAGT ACATGGTATA GGTGCTACGT ATTTACACTG GTATCAACAG AAACCAGGAA AAGCTCCGAA
TATCCCAGTG GTAGTGGACG TCCAGTTCAG TTTCGAATCA TGTACCATAT CCACGATGCA TAAATGTGAC CATAGTTGTC TTTGGTCCTT TTCGAGGCTT
18 R V T I T C R S S Q S L V H G I G A T Y L H W Y Q Q K P G K A P K

FIG. 48M

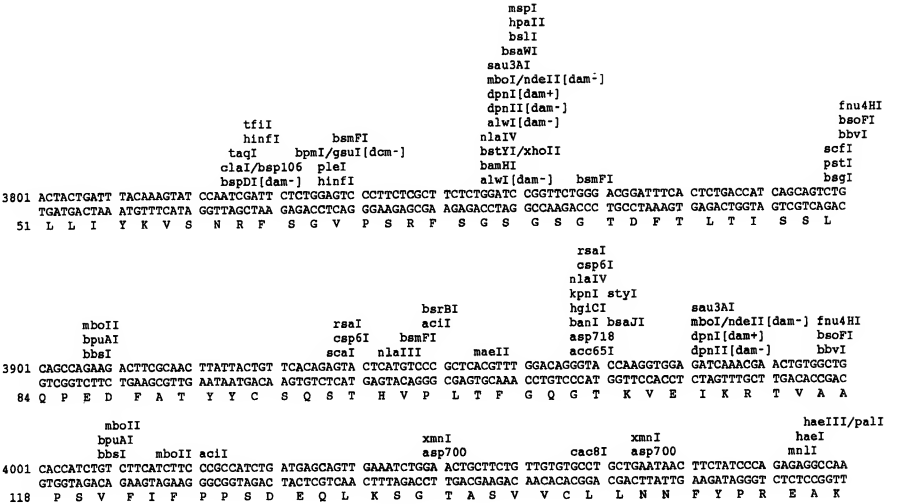

mspI
hpaII
bslI
bsaWI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
nlaIV
bstYI/xhoII
bamHI
alwI[dam-]
fnu4HI
bsoFI
bbvI
scfI
pstI
bsgI
tfiI
hinfI
bsmFI
taqI
bpmI/gsuI[dcm-]
claI/bsp106
pleI
hinfI
bspDI[dam-]
bsmFI
3801 ACTACTGATT TACAAAGTAT CCAATCGATT CTCTGGAGTC CCTTCTCGCT TCTCTGGATC CGGTTCTGGG ACGGATTTCA CTCTGACCAT CAGCAGTCTG
TGATGACTAA ATGTTTCATA GGTTAGCTAA GAGACCTCAG GGAAGAGCGA AGAGACCTAG GCCAAGACCC TGCCTAAAGT GAGACTGGTA GTCGTCAGAC
51 L L I Y K V S N R F S G V P S R F S G S G S G T D F T L T I S S L
rsaI
csp6I
nlaIV
kpnI styI
hgiCI
sau3AI
banI bsaJI
mboI/ndeII[dam-]
asp718
dpnI[dam+]
dpnII[dam-]
fnu4HI
bsoFI
bbvI
bsrBI
aciI
bsmFI
acc65I
mboII
bpuAI
bbsI
rsaI
csp6I
scaI
nlaIII
maeII
3901 CAGCCAGAAG ACTTCGCAAC TTATTACTGT TCACAGAGTA CTCATGTCCC GCTCACGTTT GGACAGGGTA CCAAGGTGGA GATCAAACGA ACTGTGGCTG
GTCGGTCTTC TGAAGCGTTG AATAATGACA AGTGTCTCAT GAGTACAGGG CGAGTGCAAA CCTGTCCCAT GGTTCCACCT CTAGTTTGCT TGACACCGAC
84 Q P E D F A T Y Y C S Q S T H V P L T F G Q G T K V E I K R T V A A
haeIII/palI
haeI
mnlI
xmnI
asp700
cac8I
mboII
bpuAI
bbsI
mboII aciI
4001 CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA ACTGCTTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
GTGGTAGACA GAAGTAGAAG GGCGGTAGAC TACTCGTCAA CTTTAGACCT TGACGAAGAC AACACACGGA CGACTTATTG AAGATAGGGT CTCTCCGGTT
118 P S V F I F P P S D E Q L K S G T A S V V C L L N N F Y P R E A K

FIG. 48N

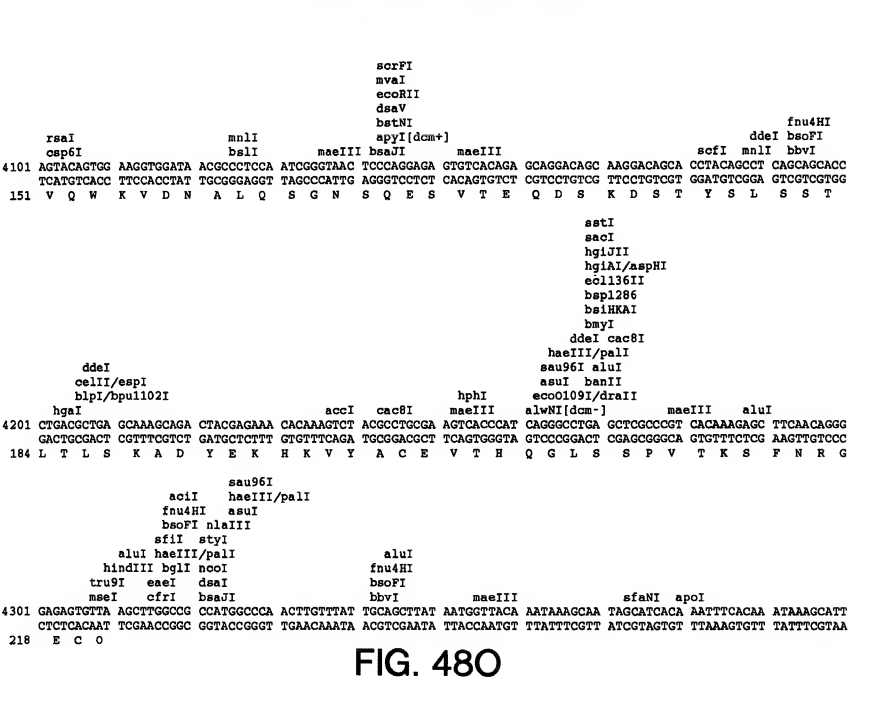

scrFI
mvaI
ecoRII
dsaV
bstNI
fnu4HI
rsaI
mnlI
apyI[dcm+]
ddeI bsoFI
csp6I
bslI
maeIII bsaJI
maeIII
scfI mnlI bbvI
4101 AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
TCATGTCACC TTCCACCTAT TGCGGGAGGT TAGCCCATTG AGGGTCCTCT CACAGTGTCT CGTCCTGTCG TTCCTGTCGT GGATGTCGGA GTCGTCGTGG
151 V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
bmyI
ddeI cac8I
haeIII/palI
sau96I aluI
asuI banII
ddeI
celII/espI
blpI/bpu1102I
hphI
eco0109I/draII
hgaI
accI cac8I maeIII alwNI[dcm-] maeIII aluI
4201 CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
GACTGCGACT CGTTTCGTCT GATGCTCTTT GTGTTTCAGA TGCGGACGCT TCAGTGGGTA GTCCCGGACT CGAGCGGGCA GTGTTTCTCG AAGTTGTCCC
184 L T L S K A D Y E K H K V Y A C E V T H Q G L S S P V T K S F N R G
sau96I
aciI haeIII/palI
fnu4HI asuI
bsoFI nlaIII
sfiI styI
aluI haeIII/palI
aluI
hindIII bglI ncoI
fnu4HI
tru9I eaeI dsaI
bsoFI
mseI cfrI bsaJI
bbvI maeIII sfaNI apoI
4301 GAGAGTGTTA AGCTTGGCCG CCATGGCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT
CTCTCACAAT TCGAACCGGC GGTACCGGGT TGAACAAATA ACGTCGAATA TTACCAATGT TTATTTCGTT ATCGTAGTGT TTAAAGTGTT TATTTCGTAA
218 E C O

FIG. 48O

```
                                                
```

nlaIII
styI
ncoI
aciI
aciI
bslI dsaI
bsmFI
aciI fokI
aciI bsrI aciI
aciI bsaJI
4701 ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT
TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA
rmaI
maeI
styI
fnu4HI
bsaJI
bsoFI
blnI
bglI
avrII[dam-]
sfiI
haeIII/palI
haeIII/palI
mnlI
mnlI
ddeI
mnlI
stuI
haeIII/palI bsaJI mnlI
aluI
mnlI
haeI
maeIII
mnlI bsaJI aciI
haeIII/palI
bseRI
mnlI bfaI
aluI
4801 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTG
AAAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAC
start pUC118^
fnu4HI
haeIII/palI
hinPI
mcrI
hhaI/cfoI
eagI/xmaIII/eclXI thaI
eaeI
fnuDII/mvnI
notI
bstUI
scrFI
bsrBI bsoFI
hinPI
bspMI
mvaI
taqI cfrI
hhaI/cfoI
scfI
ecoRII
xhoI fnu4HI tru9I
cac8I
tru9I pstI
haeIII/palI
dsaV
paeR7I bsiEI pacI
ascI
ahaIII/draI
eaeI
bstNI
avaI bsoFI
mseI tru9I bsh1236I mseI
bsgI maeIII
cfrI
bsrI
apyI[dcm+]
mnlI aciI aciI
mseI bssHII swaI
sse8387I
aluI
bsrI
maeII maeIII
bsaJI
4901 TTACCTCGAG CGGCCGCTTA ATTAAGGCGC GCCATTTAAA TCCTGCAGGT AACAGCTTGG CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC
AATGGAGCTC GCCGGCGAAT TAATTCCGCG CGGTAAATTT AGGACGTCCA TTGTCGAACC GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG
^linearization linker inserted into HpaI site

FIG. 48Q

sau3AI
mboI/ndeII[dam-]
sau96I dpnI[dam+]
cac8I haeIII/palI
aluI asuI dpnII[dam-]
fnu4HI pvuII mnlI aciI pvuI/bspCI
tru9I bsoFI nspBII mboII cac8I mcrI
maeIII mseI bbvI fokI cac8I earI/ksp632I bsiEI
5001 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT
ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA
hinPI
hhaI/cfoI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII aciI
banI sfaNI
hinPI
thaI
fnuDII/mvnI
bstUI scfI
bsh1236I
rsaI hhaI/cfoI
csp6I bslI
bglI ahaII/bsaHI sfaNI aciI aciI maeII
5101 AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG
TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC
aciI
fnu4HI fnu4HI
bsoFI bsoFI
thaI hinPI hinPI
fnuDII/mvnI hhaI/cfoI hhaI/cfoI
hinPI bstUI thaI rmaI
hhaI/cfoI hinPI fnuDII/mvnI hinPI haeII
fnu4HI hhaI/cfoI bstUI hhaI/cfoI bsrBI
bsoFI tru9I aciI bsh1236I aciI haeII maeI aciI
aciI mseI bsh1236I maeIII bbvI maeIII cac8I bfaI cac8I mboII
5201 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC
ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG

FIG. 48R

mspI
hpaII
naeI
cfr10I/bsrFI
maeII cac8I
aluI
nlaIV
hgiJII
bsp1286
bmyI
banII
nlaIV
nlaIV
hgiCI taqI
banI mnlI
5301 TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC
AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG
maeII haeIII/palI
draIII sau96I
hphI bsaAI asuI
maeII pleI
drdI hinfI maeII
tru9I
mseI
pleI
hinfI
5401 TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT
AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA
tru9I
mseI
tru9I
bslI
bsrI bslI avaI
haeIII/palI
aluI
mseI
5501 CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA
GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT
hgiAI/aspHI
bsp1286
thaI
fnuDII/mvnI
maeII
bsiHKAI
aciI
tru9I apoI tru9I
psp1406I
bmyI ddeI
fnu4HI
mseI bstUI mseI
tru9I
apaLI/snoI rsaI
bsoFI
tru9I
apoI
bsh1236I
sspI mseI
alw44I/snoI csp6I
sfaNI
mseI
aciI
5601 CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC
GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG
hinPI
hhaI/cfoI
sfaNI
mspI
hpaII
thaI
hinPI
fnu4HI
fnuDII/mvnI
scrFI
bstUI
nciI
maeIII
bsoFI
maeII bsrI nlaIII hhaI/cfoI
nspBII bsh1236I
dsaV fokI
bsaAI tth111I/aspI bbvI
aciI
aciI hgaI
drdI
cauII aciI
5701 GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA
CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT

FIG. 48S

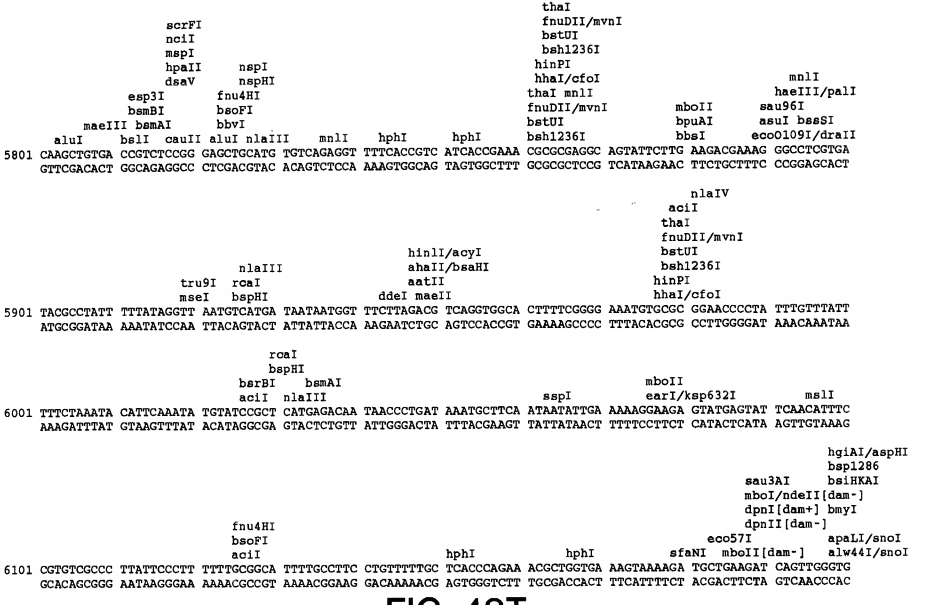

thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
thaI mnlI
fnuDII/mvnI
bstUI
bsh1236I
scrFI
nciI
mspI
hpaII
dsaV
nspI
nspHI
esp3I
bsmBI
fnu4HI
bsoFI
bbvI
maeIII bsmAI
aluI
bslI
cauII aluI nlaIII
mnlI
hphI
hphI
mnlI
haeIII/palI
mboII
sau96I
bpuAI
asuI bssSI
bbsI
eco0109I/draII
nlaIV
aciI
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
hinlI/acyI
ahaII/bsaHI
aatII
ddeI maeII
nlaIII
rcaI
tru9I
mseI
bspHI
5801 CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA
GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT
5901 TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT
ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA
roaI
bspHI
bsrBI
aciI
bsmAI
nlaIII
sspI
mboII
earI/ksp632I
mslI
6001 TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC
AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG
hgiAI/aspHI
bspl286
sau3AI
bsiHKAI
mboI/ndeII[dam-]
dpnI[dam+]
bmyI
dpnII[dam-]
fnu4HI
bsoFI
aciI
eco57I
apaLI/snoI
hphI
hphI
sfaNI
mboII[dam-]
alw44I/snoI
6101 CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG
GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC

FIG. 48T

sau3AI nspBII sau3AI
mboI/ndeII[dam-] mboI/ndeII[dam-] maeII
dpnI[dam+] dpnI[dam+] psp1406I hgiAI/aspHI
bstYI/xhoII dpnII[dam-] xmnI bsp1286 tru9I
bsrI dpnII[dam-] alwI[dam-] asp700 bsiHKAI maeI
bssSI maeIII taqI alwI[dam-] aciI bstYI/xhoII mboII bmyI ahaIII/draI
6201 CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT
GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA
scrFI
aciI nciI
thaI mspI
fnuDII/mvnI hpaII
bstUI dsaV
bsh1236I hinlI/acyI aciI rsaI
hinPI hgaI cauII mcrI fnu4HI csp6I bsrI
hhaI/cfoI ahaII/bsaHI bcgI bsiEI bsoFI ddeI scaI hphI maeIII
6301 GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC
CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG
sau3AI
haeIII/palI mboI/ndeII[dam-]
eaeI dpnI[dam+]
cfrI dpnII[dam-]
fnu4HI fnu4HI pvuI/bspCI
bsoFI bsoFI mcrI
sfaNI fokI nlaIII bbvI mslI nlaIII aciI bsiEI
6401 ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA
TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT
nlaIII
sau3AI maeIII mspI
sau96I mboI/ndeII[dam-] sau3AI nlaIV
avaII dpnI[dam+] mboI/ndeII[dam-] aluI
asuI dpnII[dam-] dpnI[dam+] hpaII
mnlI aluI aciI nlaIII alwI[dam-] dpnII[dam-] bsaWI
6501 TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA
AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT

FIG. 48U

hinPI
hhaI/cfoI
mspI
hpaII
fnu4HI
mstI
aviII/fspI
bsrI
aluI
scrFI
bsoFI
rmaI
nciI
tru9I
mslI
cac8I
bsrDI
maeII
tru9I
maeI
dsaV
mseI
maeIII
sfaNI
bbvI
psp1406I
mseI
bfaI
cauII
aseI/asnI/vspI
6601 CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA
GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT
bglI
mspI
sau96I
cac8I
hpaII
sau96I
haeIII/palI
cfr10I/bsrFI
fokI
aciI
avaII
hinPI
asuI
mspI
nlaIV hphI
bsmAI
bsrI
mnlI
asuI
hhaI/cfoI
hpaII
bpmI/gsuI[dcm-]
bsaI
6701 GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT
CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA
aciI
thaI
fnu4HI
haeIII/palI
fnuDII/mvnI
bsoFI
sau96I
pleI
hinfI
bstUI
bbvI
nlaIV
bsh1236I
bsrDI
bsrI
asuI
mnlI
ahdI/eam1105I
fokI
6801 CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG
GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC
ddeI
sau3AI
nlaIV
mboI/ndeII[dam-]
tru9I
dpnI[dam+]
hgiCI
tru9I
mseI
tru9I
dpnII[dam-]
banI
mnlI
mseI
maeIII
ahaIII/draI
mseI
6901 ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA
TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT
rmaI
maeI
sau3AI
sau3AI hphI
mboI/ndeII[dam-]
mboI/ndeII[dam-]
dpnI[dam+]
dpnI[dam+]
dpnII[dam-]
dpnII[dam-]
sau3AI
tru9I bstYI/xhoII
alwI[dam-]
nlaIII
maeII
mboI/ndeII[dam-]
mseI alwI[dam-]
bstYI/xhoII
rcaI
tru9I
hgaI
dpnI[dam+]
ahaIII/draI bfaI
mboII[dam-]
bspHI
mseI
ddeI
dpnII[dam-]
7001 TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA
AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT

FIG. 48V

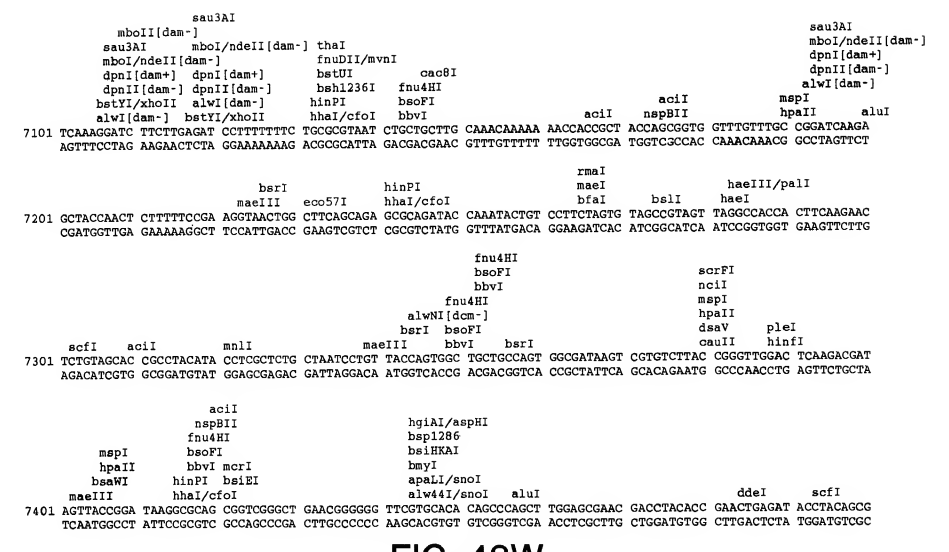

sau3AI
mboII[dam-]
sau3AI mboI/ndeII[dam-] thaI
mboI/ndeII[dam-] fnuDII/mvnI
dpnI[dam+] dpnI[dam+] bstUI cac8I
dpnII[dam-] dpnII[dam-] bsh1236I fnu4HI
bstYI/xhoII alwI[dam-] hinPI bsoFI aciI mspI
alwI[dam-] bstYI/xhoII hhaI/cfoI bbvI aciI nspBII hpaII aluI
7101 TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA
AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
rmaI
maeI haeIII/palI
bsrI hinPI bfaI bslI haeI
maeIII eco57I hhaI/cfoI
7201 GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC
CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG
fnu4HI
bsoFI scrFI
bbvI nciI
fnu4HI mspI
alwNI[dcm-] hpaII
bsrI bsoFI dsaV pleI
scfI aciI mnlI maeIII bbvI bsrI cauII hinfI
7301 TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT
AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA
aciI
nspBII hgiAI/aspHI
fnu4HI bsp1286
mspI bsoFI bsiHKAI
hpaII bbvI mcrI bmyI
bsaWI hinPI bslEI apaLI/snoI
maeIII hhaI/cfoI alw44I/snoI aluI ddeI scfI
7401 AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG
TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC

FIG. 48W

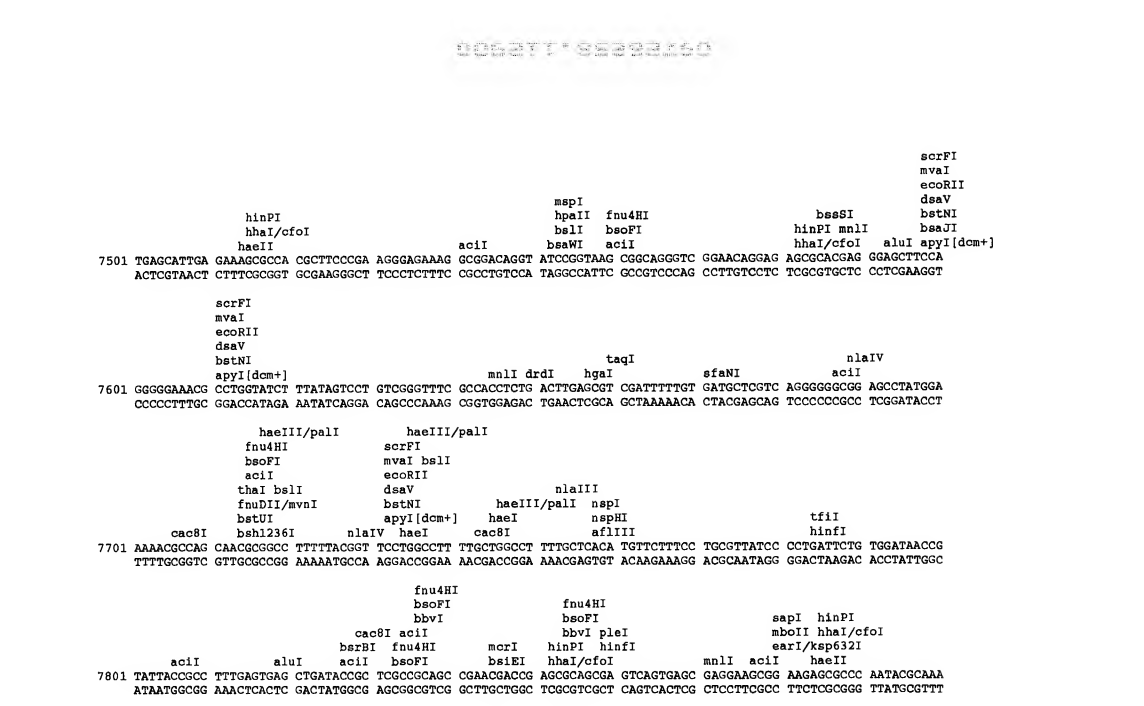

scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
mspI
hpaII fnu4HI
bslI bsoFI
bsaWI aciI
bssSI
hinPI mnlI
hhaI/cfoI
aluI
hinPI
hhaI/cfoI
haeII
aciI
7501 TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA
ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
taqI
mnlI drdI
hgaI
sfaNI
nlaIV
aciI
7601 GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA
CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA GCTAAAAACA CTACGAGCAG TCCCCCGCC TCGGATACCT
haeIII/palI
fnu4HI
bsoFI
aciI
thaI bslI
fnuDII/mvnI
bstUI
cac8I
bsh1236I
haeIII/palI
scrFI
mvaI bslI
ecoRII
dsaV
bstNI
apyI[dcm+]
nlaIII
haeIII/palI
haeI
cac8I
nlaIV
haeI
nspI
nspHI
aflIII
tfiI
hinfI
7701 AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG
TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC
fnu4HI
bsoFI
bbvI
cac8I aciI
bsrBI fnu4HI
aciI
aluI
aciI bsoFI
mcrI
bsiEI
fnu4HI
bsoFI
bbvI pleI
hinPI hinfI
hhaI/cfoI
sapI hinPI
mboII hhaI/cfoI
earI/ksp632I
mnlI aciI
haeII
7801 TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA
ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT

FIG. 48X

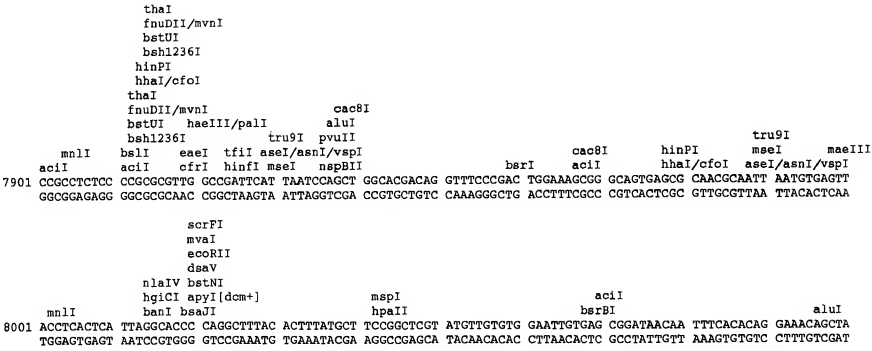

thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
cac8I
bstUI
haeIII/palI
aluI
bsh1236I
tru9I
pvuII
tru9I
mnlI
bslI
eaeI
tfiI
aseI/asnI/vspI
cac8I
hinPI
mseI
maeIII
aciI
aciI
cfrI
hinfI
mseI
nspBII
bsrI
aciI
hhaI/cfoI
aseI/asnI/vspI
7901 CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT
GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA
scrFI
mvaI
ecoRII
dsaV
nlaIV bstNI
hgiCI apyI[dcm+]
mspI
aciI
mnlI
banI bsaJI
hpaII
bsrBI
aluI
8001 ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA
TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT

FIG. 48Y

```
                    tru9I
                    mseI
                   aseI/asnI/vspI
                  xmnI
          nlaIII    asp700
  8101 TGACCATGAT TACGAATTAA
       ACTGGTACTA ATGCTTAATT

>length: 8120

aatII(GACGTC):          1690 5947
acc65I(GGTACC):         2969 3967 4529
accI(GTMKAC):           823 1039 2738 4237
aciI(CCGC):             217 229 238 250 260 271 317 422 454 485 574 1385 1795 1871 2248 2250 2758 2982
                        3167 3179 3188 3200 3210 3221 3267 3372 3404 3449 3686 3949 4021 4318 4542 4727
                        4739 4748 4760 4770 4781 4827 4910 4914 5070 5127 5153 5166 5203 5217 5220 5248
                        5275 5680 5699 5741 5751 5790 5979 6026 6125 6234 6311 6355 6476 6522 6713 6804
                        7166 7175 7310 7420 7541 7560 7687 7715 7806 7827 7834 7877 7901 7911 7967 8070
acyI                    see hinlI
aflII/bfrI(CTTAAG):     786
aflIII(ACRYGT):         932 7758
ageI(ACCGGT):           1833
ahaII/bsaHI(GRCGYC):    988 1690 1858 5117 5947 6329
ahaIII/draI(TTTAAA):    696 4935 6290 6982 7001
ahdI/eam1105I(GACNNNNNGTC): 2087 6865
aluI(AGCT):             5 44 332 386 390 753 1097 1165 1370 1431 1951 2603 2751 2784 3282 3336 3340
                        3562 3566 3676 3733 3792 4270 4288 4311 4344 4554 4842 4896 4954 5047 5333 5590
                        5803 5822 6516 6579 6679 7200 7457 7593 7819 7937 8096
alw44I/snoI(GTGCAC):    1876 5651 6198 7444
```

FIG. 48Z

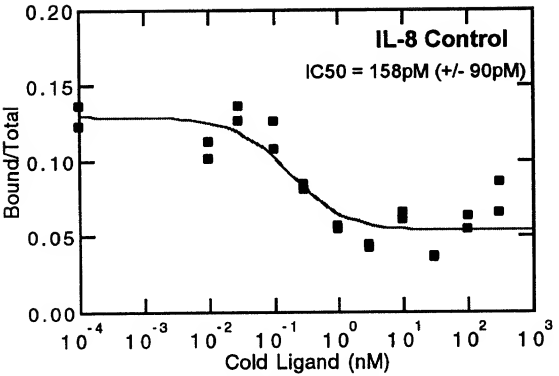


FIG. 49A

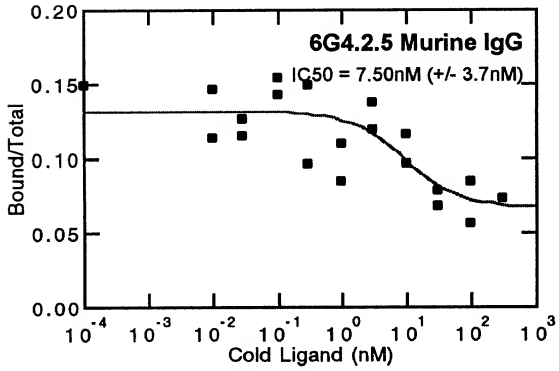


FIG. 49B

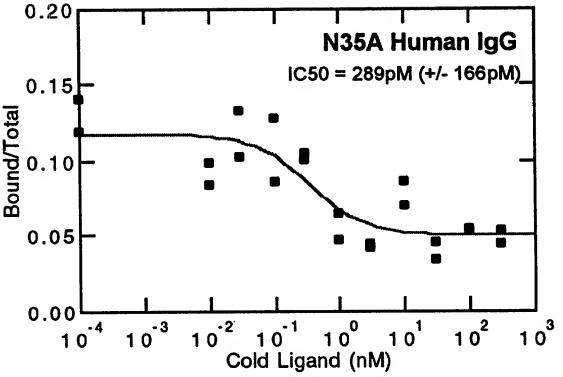


FIG. 49C

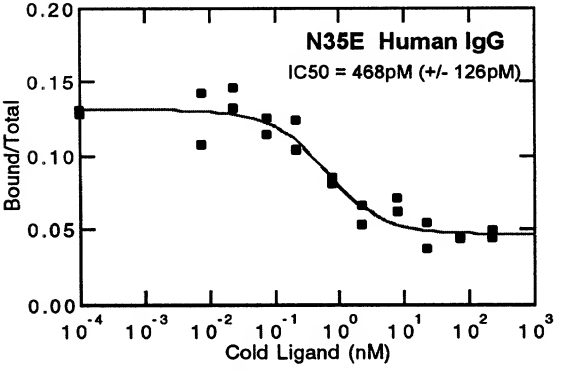


FIG. 49D

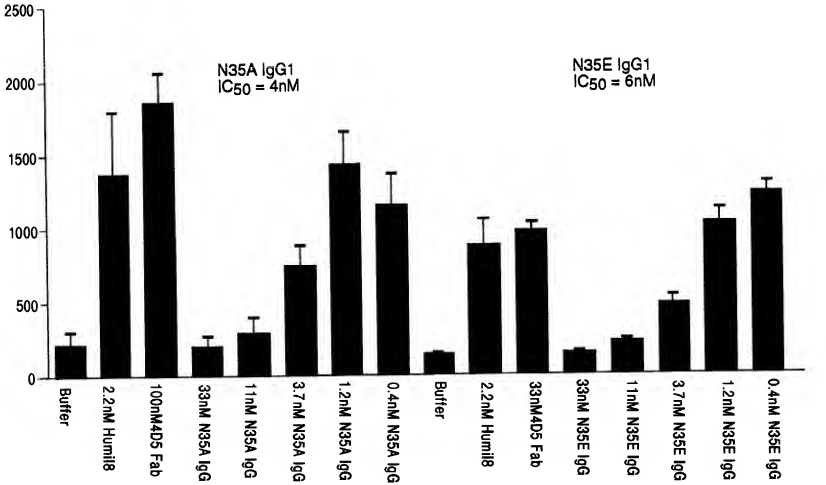

2500
2000
1500
1000
500
0
N35A IgG1
IC50 = 4nM
N35E IgG1
IC50 = 6nM
Buffer
2.2nM Humil8
100nM4D5 Fab
33nM N35A IgG
11nM N35A IgG
3.7nM N35A IgG
1.2nM N35A IgG
0.4nM N35A IgG
Buffer
2.2nM Humil8
33nM4D5 Fab
33nM N35E IgG
11nM N35E IgG
3.7nM N35E IgG
1.2nM N35E IgG
0.4nM N35E IgG

FIG. 50A

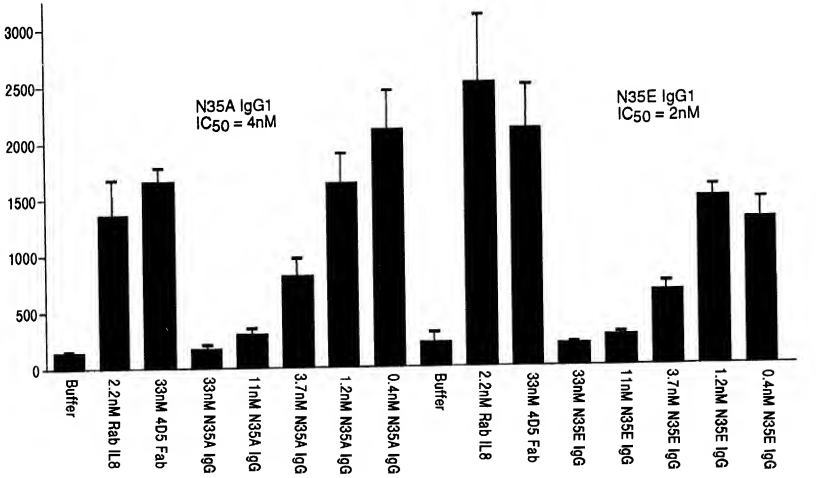
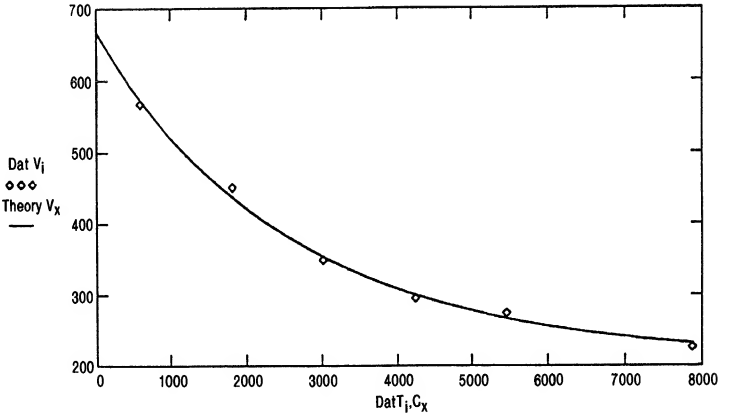

3000
2500
2000
1500
1000
500
0
N35A IgG1
IC50 = 4nM
N35E IgG1
IC50 = 2nM
Buffer
2.2nM Rab IL8
33nM 4D5 Fab
33nM N35A IgG
11nM N35A IgG
3.7nM N35A IgG
1.2nM N35A IgG
0.4nM N35A IgG
Buffer
2.2nM Rab IL8
33nM 4D5 Fab
33nM N35E IgG
11nM N35E IgG
3.7nM N35E IgG
1.2nM N35E IgG
0.4nM N35E IgG

FIG. 50B



Representative Conc versus Time Plot. Shown is the kinetic data for 6G4V11N35A.IgG1

SAMPLE	ka	kd	Kd
Murine 6G4.2.5 IgG2a	$8.3x10^5$	$2.9x10^{-4}$	350pM
6G4V11N35A-IgG1	$8.7x10^5$	$7.7x10^{-5}$	88pM
6G4V11N35E-IgG1	$3.0x10^6$	$1.4x10^{-4}$	49pM

FIG. 51

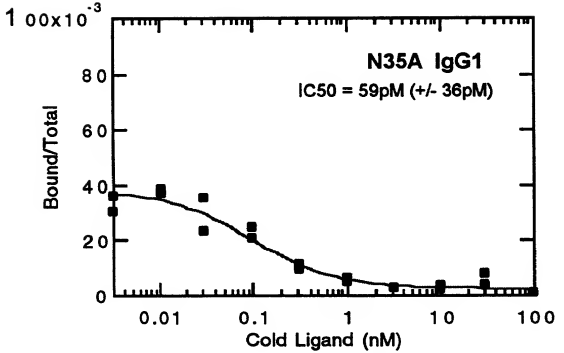


FIG. 52A

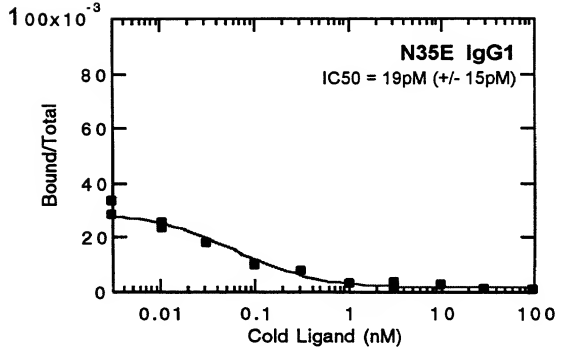


FIG. 52B

```
781 AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT TCTTCTTGCA
    TTTTCCCATA GATCTCCAAC TCCACTAAAA TACTTTTTCT TATAGCGTAA AGAAGAACGT
 -1                                   M  K  K  N    I  A  F    L  L  A

841 TCTATGTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG AGGTTCAGCT AGTGCAGTCT
    AGATACAAGC AAAAAAGATA ACGATGTTTG CGCATGCGAC TCCAAGTCGA TCACGTCAGA
-11 S  M  F  V    F  S  I    A  T  N    A  Y  A  E    V  Q  L    V  Q  S

901 GGCGGTGGCC TGGTGCAGCC AGGGGGCTCA CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC
    CCGCCACCGG ACCACGTCGG TCCCCCGAGT GAGGCAAACA GGACACGTCG AAGACCGATG
 8  G  G  G  L    V  Q  P    G  G  S    L  R  L  S    C  A  A    S  G  Y

961 TCCTTCTCGA GTCACTATAT GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG
    AGGAAGAGCT CAGTGATATA CGTGACCCAG GCAGTCCGGG GCCCATTCCC GGACCTTACC
 28 S  F  S  S    H  Y  M    H  W  V    R  Q  A  P    G  K  G    L  E  W

1021 GTTGGATATA TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT
     CAACCTATAT AACTAGGAAG GTTACCACTT TGATGCATAT TAGTTTTCAA GTTCCCGGCA
 48  V  G  Y  I    D  P  S    N  G  E    T  T  Y  N    Q  K  F    K  G  R

1081 TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA CAGCCTGCGT
     AAGTGAAATA GAGCGCTGTT GAGGTTTTTG TGTCGTATGG ACGTCTACTT GTCGGACGCA
 68  F  T  L  S    R  D  N    S  K  N    T  A  Y  L    Q  M  N    S  L  R

1141 GCTGAGGACA CTGCCGTCTA TTACTGTGCA AGAGGGGATT ATCGCTACAA TGGTGACTGG
     CGACTCCTGT GACGGCAGAT AATGACACGT TCTCCCCTAA TAGCGATGTT ACCACTGACC
 88  A  E  D  T    A  V  Y    Y  C  A    R  G  D  Y    R  Y  N    G  D  W

1201 TTCTTCGACG TCTGGGGTCA AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC
     AAGAAGCTGC AGACCCCAGT TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG
 108 F  F  D  V    W  G  Q    G  T  L    V  T  V  S    S  A  S    T  K  G

1261 CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCGTG TCGCCGGGAC
 128 P  S  V  F    P  L  A    P  S  S    K  S  T  S    G  G  T    A  A  L

1321 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG
 148 G  C  L  V    K  D  Y    F  P  E    P  V  T  V    S  W  N    S  G  A

1381 CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
     GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA GGAGTCCTGA GATGAGGGAG
 168 L  T  S  G    V  H  T    F  P  A    V  L  Q  S    S  G  L    Y  S  L

1441 AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG
     TCGTCGCACC ACTGGCACGG GAGGTCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC
 188 S  S  V  V    T  V  P    S  S  S    L  G  T  Q    T  Y  I    C  N  V

1501 AATCACAAGC CCAGCAACAC CAAGGTCGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA
     TTAGTGTTCG GGTCGTTGTG GTTCCAGCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT
 208 N  H  K  P    S  N  T    K  V  D    K  K  V  E    P  K  S    C  D  K

1561 ACTCACACAT GCCCGCCGTGA
     TGAGTGTGTA CGGGCGGCACT
 228 T  H  T  C    P  P  O
```

FIG. 53

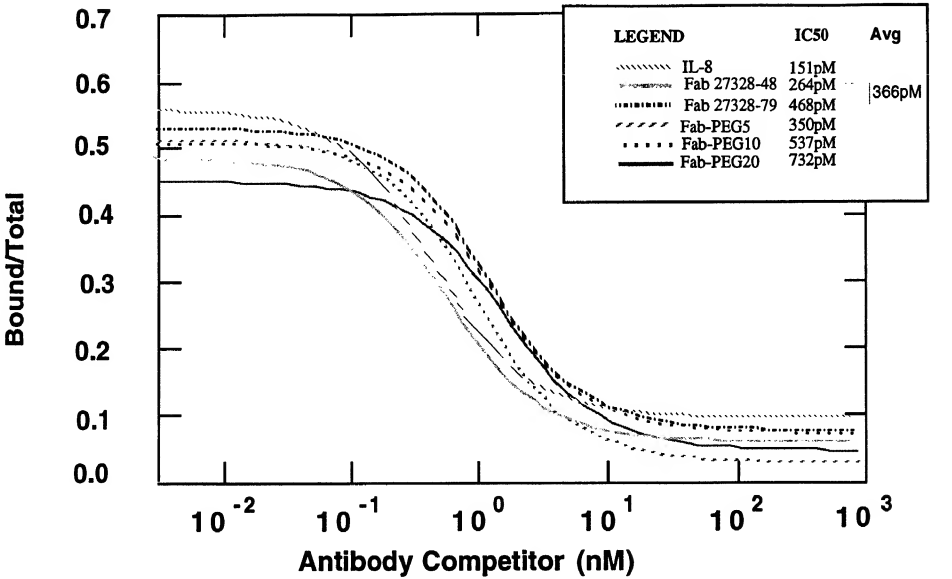

LEGEND
IC50
Avg
IL-8 151pM
Fab 27328-48 264pM
Fab 27328-79 468pM
366pM
Fab-PEG5 350pM
Fab-PEG10 537pM
Fab-PEG20 732pM
Bound/Total
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10 -2
10 -1
10 0
10 1
10 2
10 3
Antibody Competitor (nM)

FIG. 54A

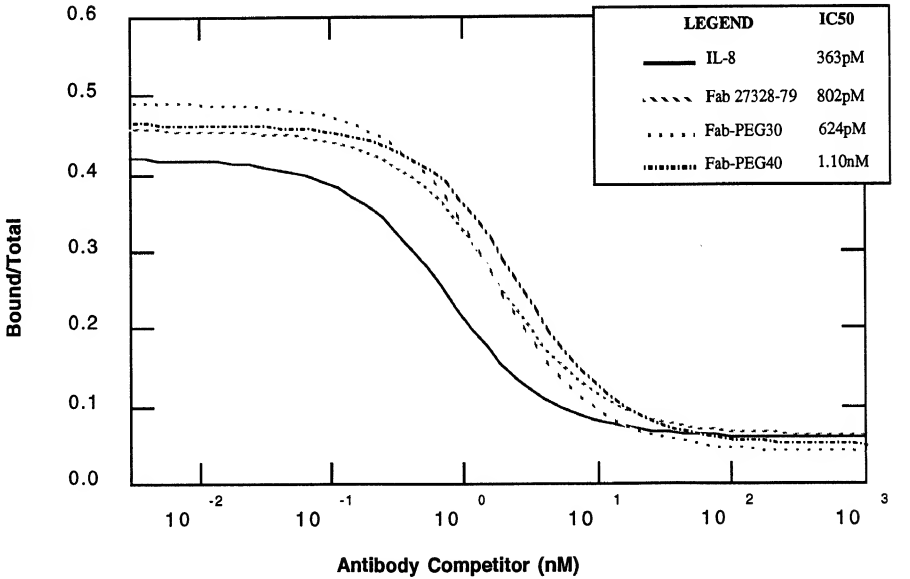

LEGEND
IC50
IL-8
363pM
Fab 27328-79
802pM
Fab-PEG30
624pM
Fab-PEG40
1.10nM
Bound/Total
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10 -2
10 -1
10 0
10 1
10 2
10 3
Antibody Competitor (nM)

FIG. 54B

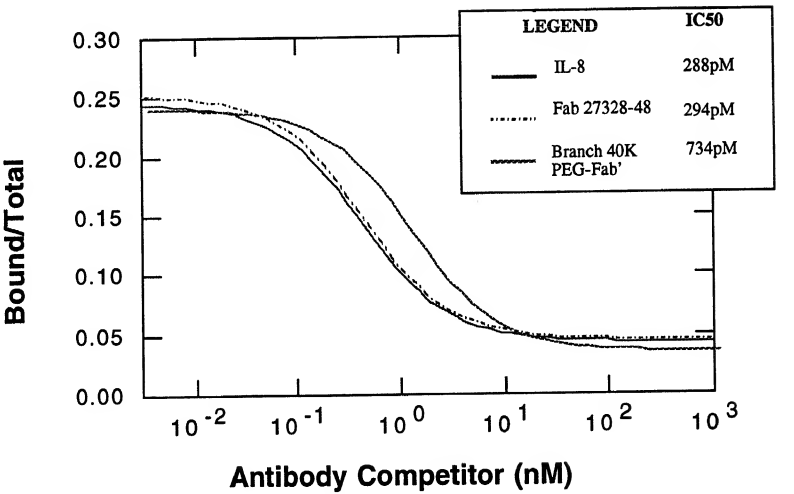

LEGEND
IC50
IL-8
288pM
Fab 27328-48
294pM
Branch 40K PEG-Fab'
734pM
Bound/Total
0.30
0.25
0.20
0.15
0.10
0.05
0.00
10 -2
10 -1
10 0
10 1
10 2
10 3
Antibody Competitor (nM)

FIG. 54C

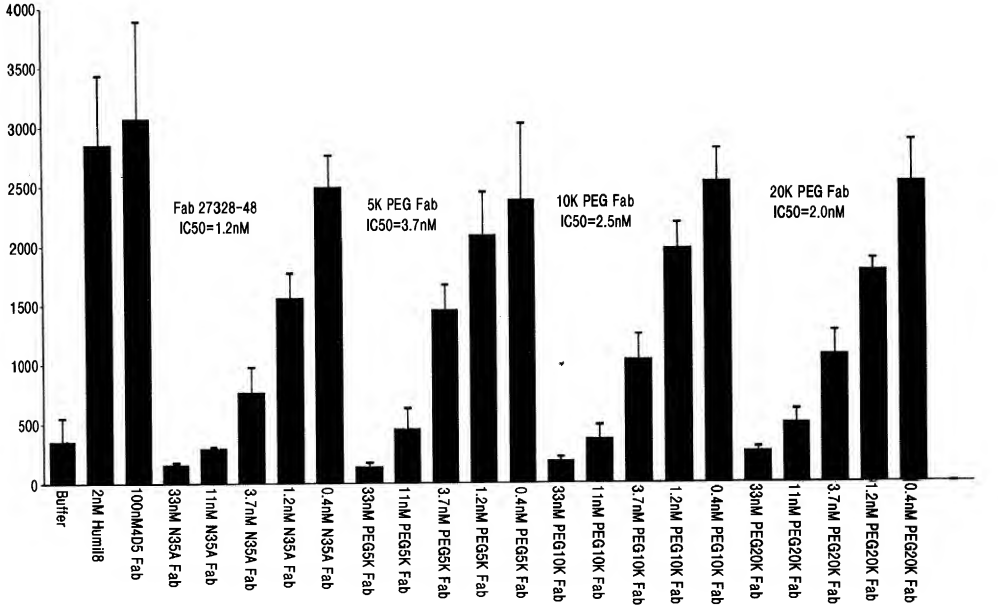

4000
3500
3000
2500
2000
1500
1000
500
0
Fab 27328-48
IC50=1.2nM
5K PEG Fab
IC50=3.7nM
10K PEG Fab
IC50=2.5nM
20K PEG Fab
IC50=2.0nM
Buffer
2nM Humil8
100nM4D5 Fab
33nM N35A Fab
11nM N35A Fab
3.7nM N35A Fab
1.2nM N35A Fab
0.4nM N35A Fab
33nM PEG5K Fab
11nM PEG5K Fab
3.7nM PEG5K Fab
1.2nM PEG5K Fab
0.4nM PEG5K Fab
33nM PEG10K Fab
11nM PEG10K Fab
3.7nM PEG10K Fab
1.2nM PEG10K Fab
0.4nM PEG10K Fab
33nM PEG20K Fab
11nM PEG20K Fab
3.7nM PEG20K Fab
1.2nM PEG20K Fab
0.4nM PEG20K Fab

FIG. 55A

DRAFT 2023

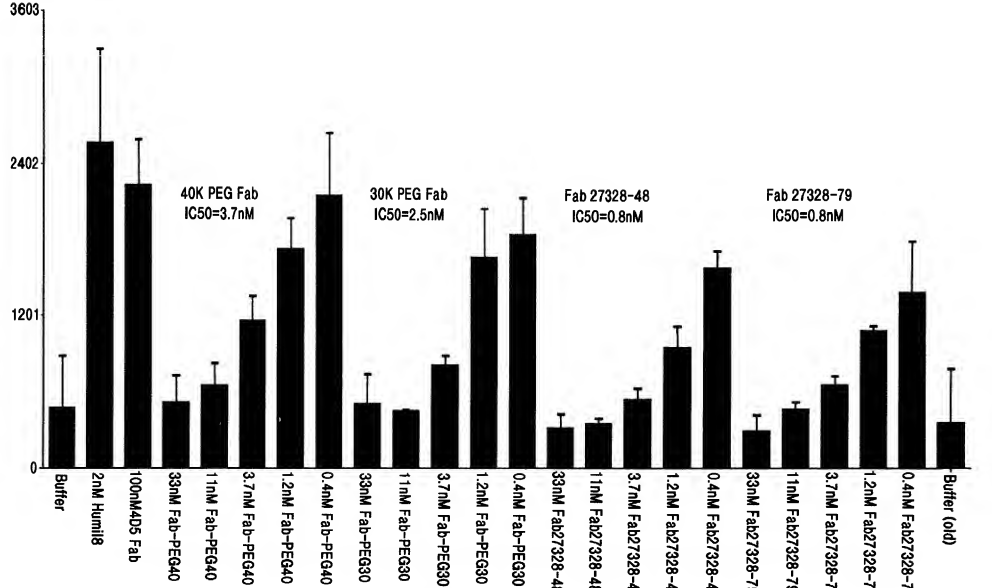


FIG. 55B

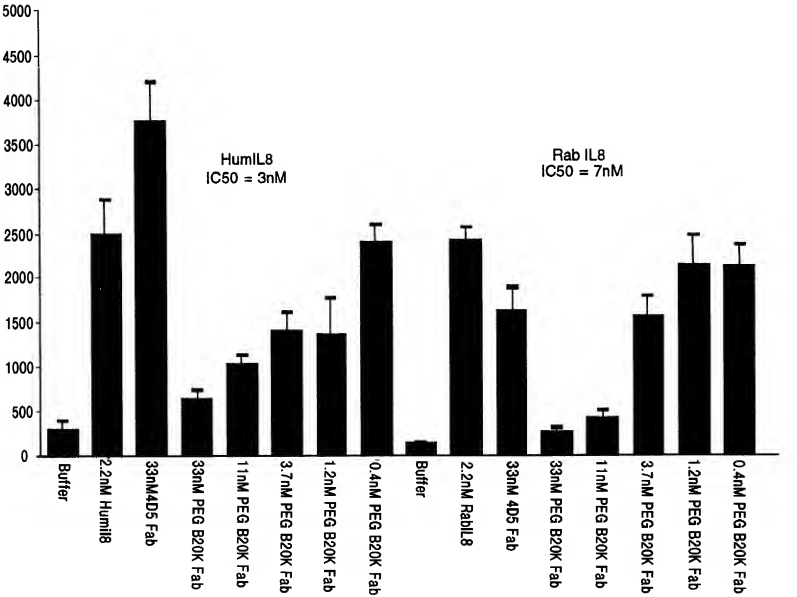

5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
HumIL8
IC50 = 3nM
Rab IL8
IC50 = 7nM
Buffer
2.2nM HumIl8
33nM4D5 Fab
33nM PEG B20K Fab
11nM PEG B20K Fab
3.7nM PEG B20K Fab
1.2nM PEG B20K Fab
0.4nM PEG B20K Fab
Buffer
2.2nM RabIL8
33nM 4D5 Fab
33nM PEG B20K Fab
11nM PEG B20K Fab
3.7nM PEG B20K Fab
1.2nM PEG B20K Fab
0.4nM PEG B20K Fab

FIG. 55C

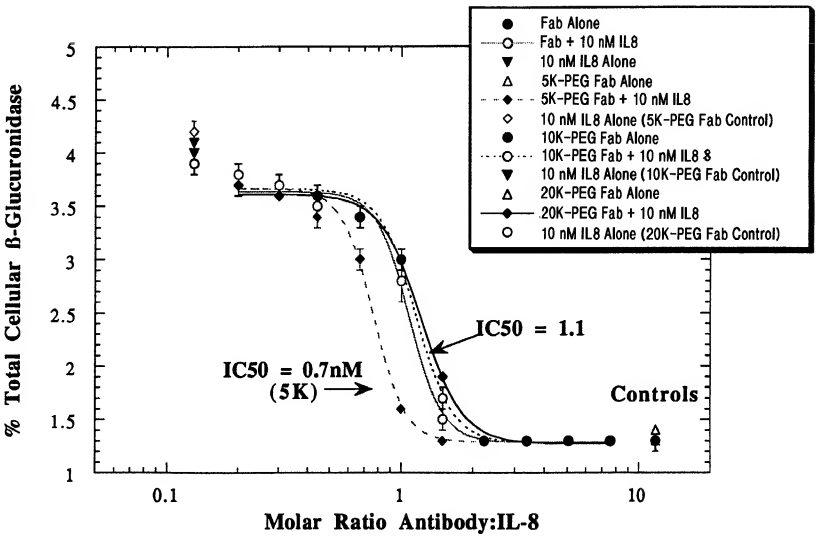

% Total Cellular ß-Glucuronidase
5
4.5
4
3.5
3
2.5
2
1.5
1
0.1
1
10
Molar Ratio Antibody:IL-8
Fab Alone
Fab + 10 nM IL8
10 nM IL8 Alone
5K-PEG Fab Alone
5K-PEG Fab + 10 nM IL8
10 nM IL8 Alone (5K-PEG Fab Control)
10K-PEG Fab Alone
10K-PEG Fab + 10 nM IL8
10 nM IL8 Alone (10K-PEG Fab Control)
20K-PEG Fab Alone
20K-PEG Fab + 10 nM IL8
10 nM IL8 Alone (20K-PEG Fab Control)
IC50 = 1.1
IC50 = 0.7nM
(5K)
Controls

FIG. 56A

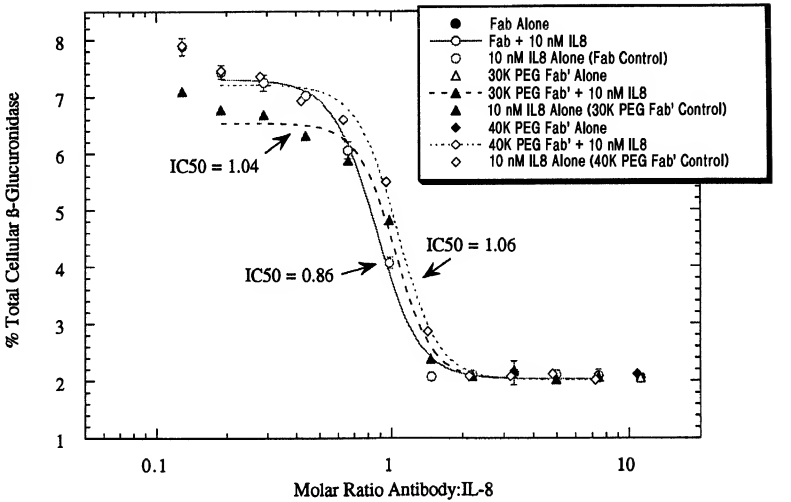

Fab Alone
Fab + 10 nM IL8
10 nM IL8 Alone (Fab Control)
30K PEG Fab' Alone
30K PEG Fab' + 10 nM IL8
10 nM IL8 Alone (30K PEG Fab' Control)
40K PEG Fab' Alone
40K PEG Fab' + 10 nM IL8
10 nM IL8 Alone (40K PEG Fab' Control)
IC50 = 1.04
IC50 = 0.86
IC50 = 1.06
% Total Cellular ß-Glucuronidase
Molar Ratio Antibody:IL-8
8
7
6
5
4
3
2
1
0.1
1
10

FIG. 56B

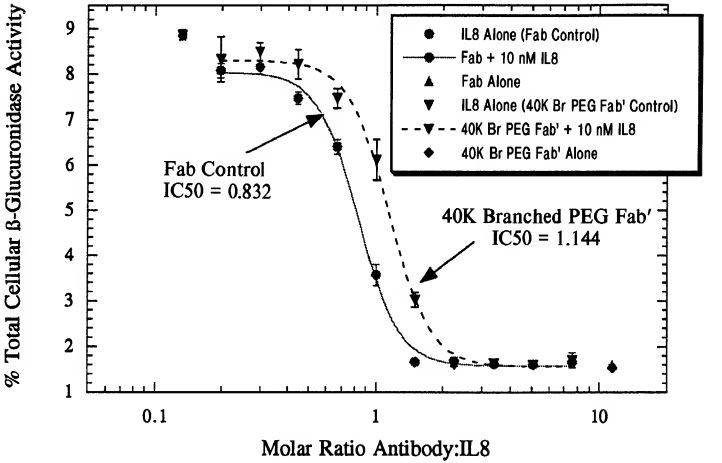

% Total Cellular ß-Glucuronidase Activity
9
8
7
6
5
4
3
2
1
0.1
1
10
Molar Ratio Antibody:IL8
IL8 Alone (Fab Control)
Fab + 10 nM IL8
Fab Alone
IL8 Alone (40K Br PEG Fab' Control)
40K Br PEG Fab' + 10 nM IL8
40K Br PEG Fab' Alone
Fab Control
IC50 = 0.832
40K Branched PEG Fab'
IC50 = 1.144

FIG. 56C

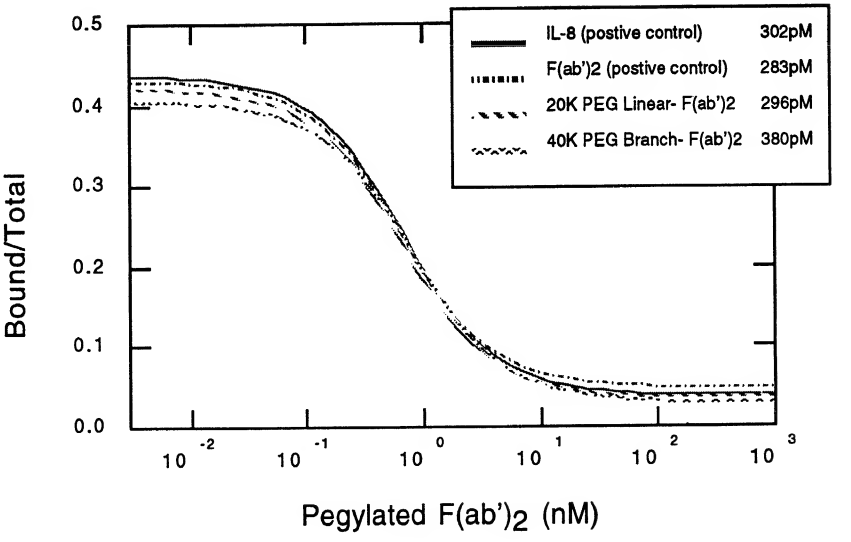

IL-8 (postive control) 302pM
F(ab')2 (postive control) 283pM
20K PEG Linear- F(ab')2 296pM
40K PEG Branch- F(ab')2 380pM
Bound/Total
0.5
0.4
0.3
0.2
0.1
0.0
10 -2
10 -1
10 0
10 1
10 2
10 3
Pegylated F(ab')2 (nM)

FIG. 57A

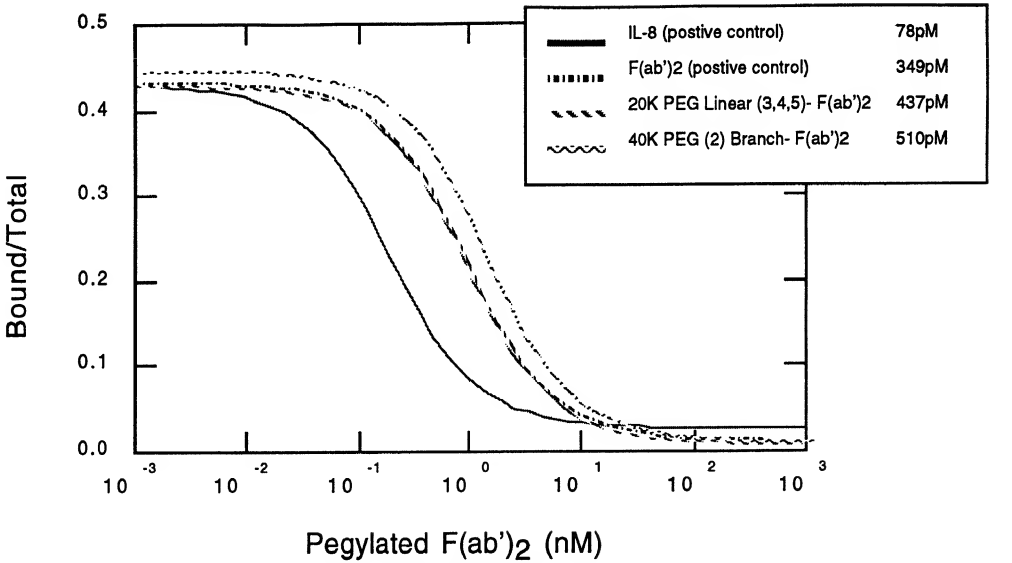

IL-8 (postive control) 78pM
F(ab')2 (postive control) 349pM
20K PEG Linear (3,4,5)- F(ab')2 437pM
40K PEG (2) Branch- F(ab')2 510pM
Bound/Total
0.5
0.4
0.3
0.2
0.1
0.0
10 -3
10 -2
10 -1
10 0
10 1
10 2
10 3
Pegylated F(ab')2 (nM)

FIG. 57B

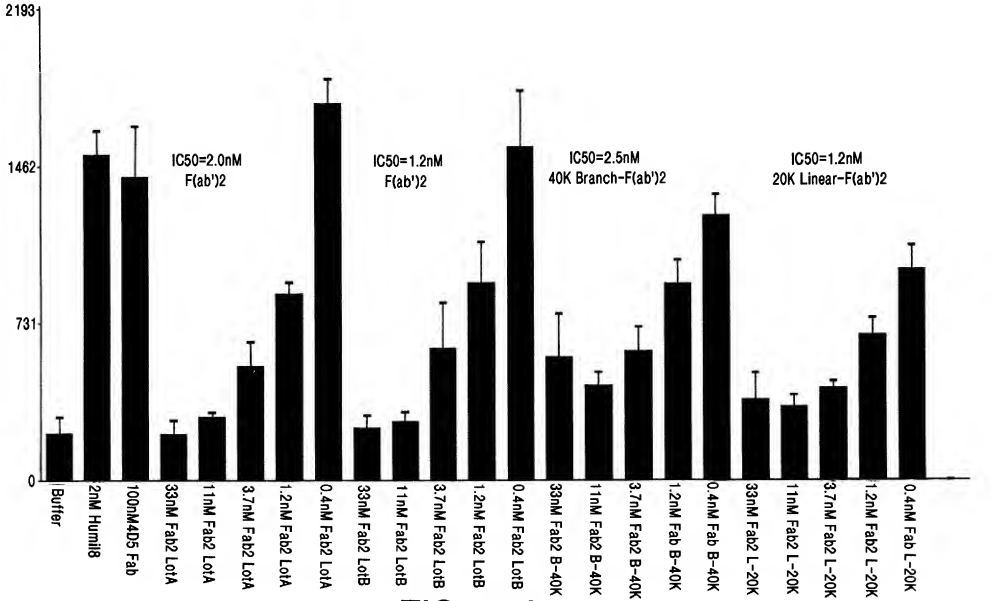

2193
1462
731
0
IC50=2.0nM
F(ab')2
IC50=1.2nM
F(ab')2
IC50=2.5nM
40K Branch-F(ab')2
IC50=1.2nM
20K Linear-F(ab')2
Buffer
2nM Humil8
100nM4D5 Fab
33nM Fab2 LotA
11nM Fab2 LotA
3.7nM Fab2 LotA
1.2nM Fab2 LotA
0.4nM Fab2 LotA
33nM Fab2 LotB
11nM Fab2 LotB
3.7nM Fab2 LotB
1.2nM Fab2 LotB
0.4nM Fab2 LotB
33nM Fab2 B-40K
11nM Fab2 B-40K
3.7nM Fab2 B-40K
1.2nM Fab B-40K
0.4nM Fab B-40K
33nM Fab2 L-20K
11nM Fab2 L-20K
3.7nM Fab2 L-20K
1.2nM Fab2 L-20K
0.4nM Fab L-20K

FIG. 58A

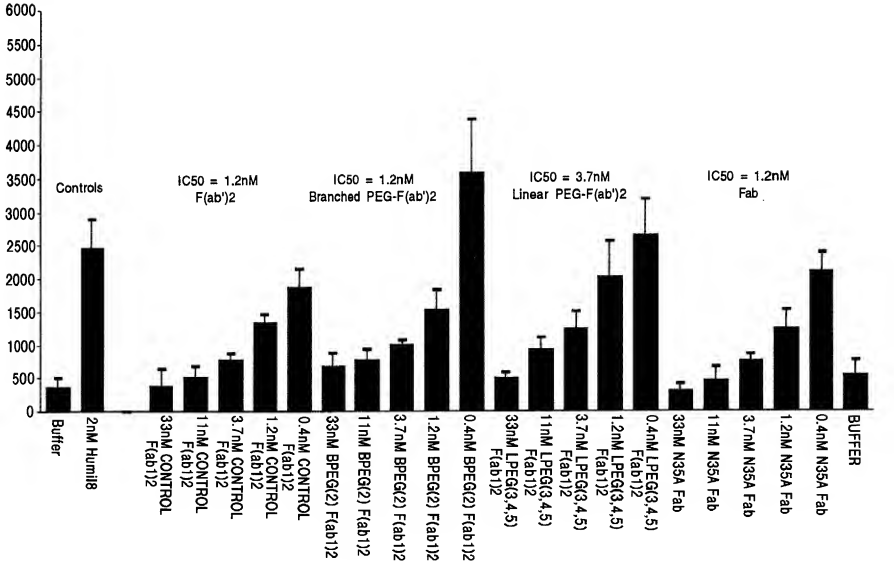

6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Controls
IC50 = 1.2nM F(ab')2
IC50 = 1.2nM Branched PEG-F(ab')2
IC50 = 3.7nM Linear PEG-F(ab')2
IC50 = 1.2nM Fab
Buffer
2nM Humil8
33nM CONTROL F(ab')2
11nM CONTROL F(ab')2
3.7nM CONTROL F(ab')2
1.2nM CONTROL F(ab')2
0.4nM CONTROL F(ab')2
33nM BPEG(2) F(ab')2
11nM BPEG(2) F(ab')2
3.7nM BPEG(2) F(ab')2
1.2nM BPEG(2) F(ab')2
0.4nM BPEG(2) F(ab')2
33nM LPEG(3,4,5) F(ab')2
11nM LPEG(3,4,5) F(ab')2
3.7nM LPEG(3,4,5) F(ab')2
1.2nM LPEG(3,4,5) F(ab')2
0.4nM LPEG(3,4,5) F(ab')2
33nM N35A Fab
11nM N35A Fab
3.7nM N35A Fab
1.2nM N35A Fab
0.4nM N35A Fab
BUFFER

FIG. 58B

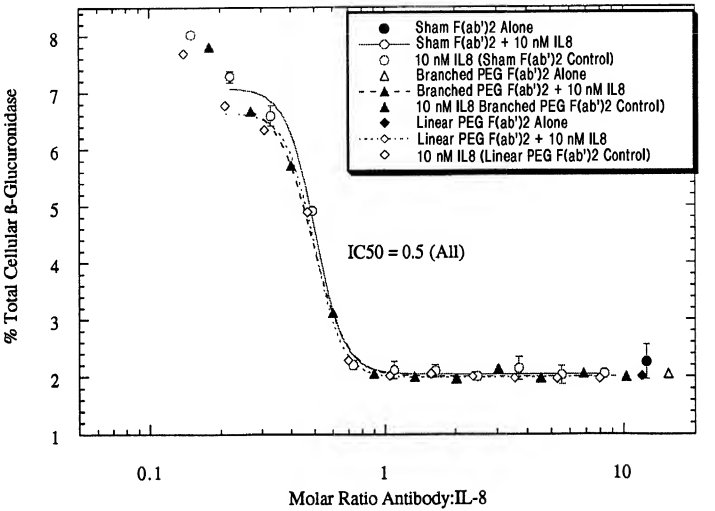

Sham F(ab')2 Alone
Sham F(ab')2 + 10 nM IL8
10 nM IL8 (Sham F(ab')2 Control)
Branched PEG F(ab')2 Alone
Branched PEG F(ab')2 + 10 nM IL8
10 nM IL8 Branched PEG F(ab')2 Control)
Linear PEG F(ab')2 Alone
Linear PEG F(ab')2 + 10 nM IL8
10 nM IL8 (Linear PEG F(ab')2 Control)
IC50 = 0.5 (All)
% Total Cellular β-Glucuronidase
Molar Ratio Antibody:IL-8
8
7
6
5
4
3
2
1
0.1
1
10

FIG. 59A

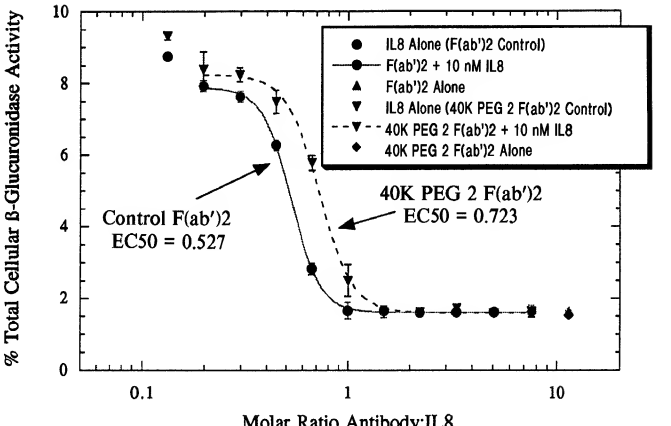

% Total Cellular ß-Glucuronidase Activity
10
8
6
4
2
0
0.1
1
10
Molar Ratio Antibody:IL8
IL8 Alone (F(ab')2 Control)
F(ab')2 + 10 nM IL8
F(ab')2 Alone
IL8 Alone (40K PEG 2 F(ab')2 Control)
40K PEG 2 F(ab')2 + 10 nM IL8
40K PEG 2 F(ab')2 Alone
Control F(ab')2
EC50 = 0.527
40K PEG 2 F(ab')2
EC50 = 0.723

FIG. 59B

CONFIDENTIAL

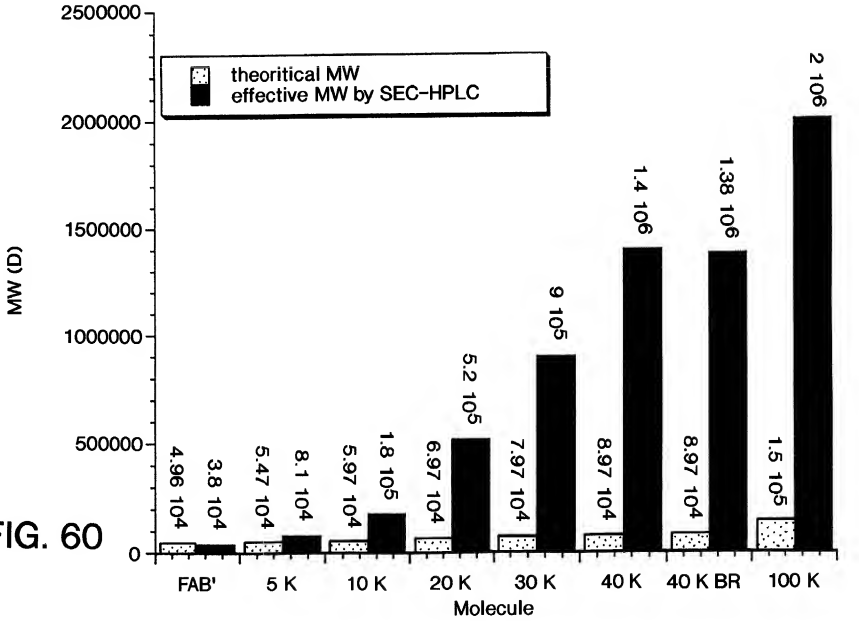


FIG. 60

FIG. 61A

FIG. 61B

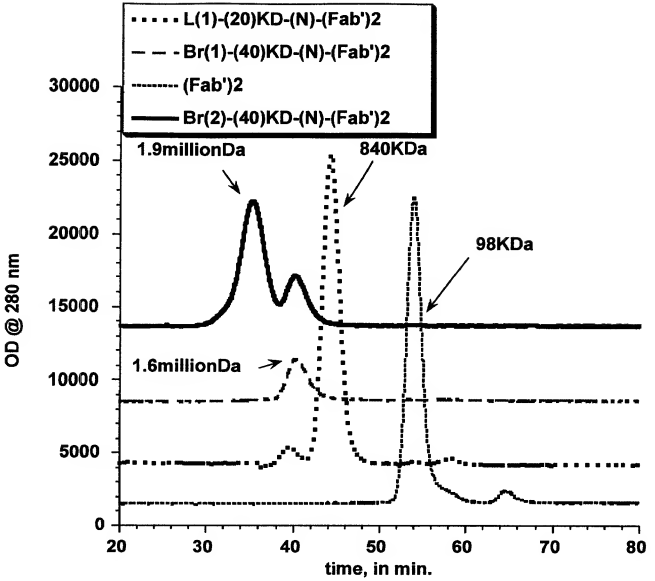

L(1)-(20)KD-(N)-(Fab')2
Br(1)-(40)KD-(N)-(Fab')2
(Fab')2
Br(2)-(40)KD-(N)-(Fab')2
1.9millionDa
840KDa
98KDa
1.6millionDa
OD @ 280 nm
time, in min.
30000
25000
20000
15000
10000
5000
0
20
30
40
50
60
70
80

FIG. 62

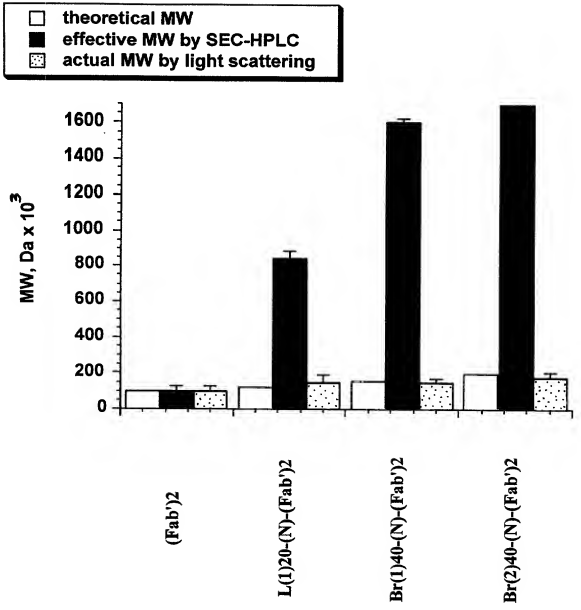

theoretical MW
effective MW by SEC-HPLC
actual MW by light scattering
MW, Da x 10^3
1600
1400
1200
1000
800
600
400
200
0
(Fab')2
L(1)20-(N)-(Fab')2
Br(1)40-(N)-(Fab')2
Br(2)40-(N)-(Fab')2

FIG. 63

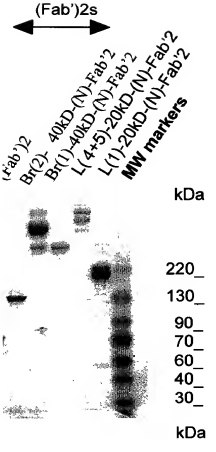

(Fab')2s
(Fab')2
Br(2)-40kD-(N)-Fab'2
Br(1)-40kD-(N)-Fab'2
L(4+5)-20kD-(N)-Fab'2
L(1)-20kD-(N)-Fab'2
MW markers
kDa
220_
130_
90_
70_
60_
40_
30_
kDa

FIG. 64

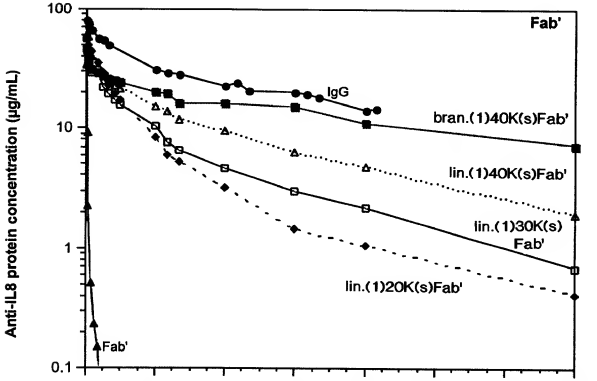


FIG. 65A

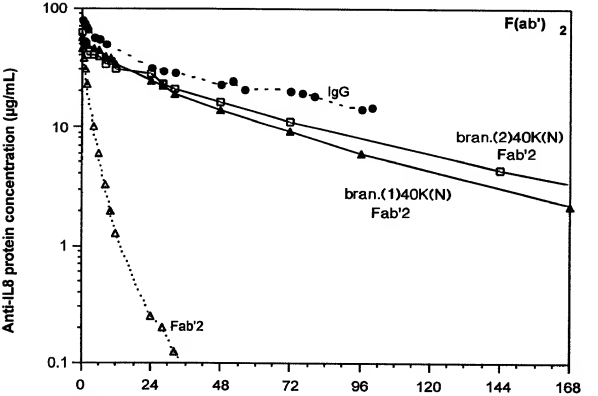


FIG. 65B

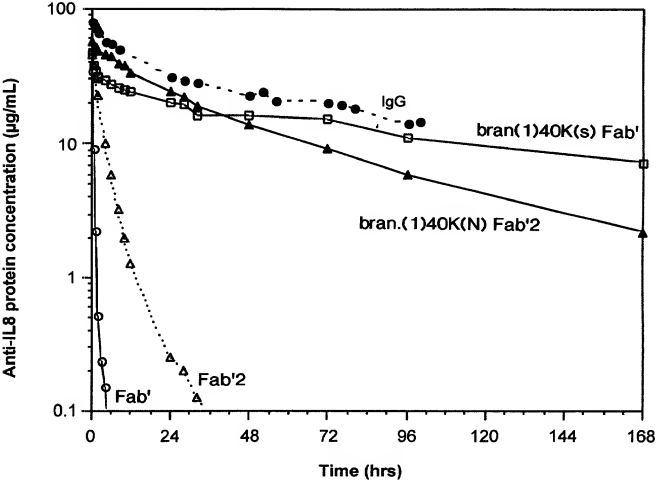

100
10
1
0.1
Anti-IL8 protein concentration (µg/mL)
0
24
48
72
96
120
144
168
Time (hrs)
IgG
bran(1)40K(s) Fab'
bran.(1)40K(N) Fab'2
Fab'2
Fab'

FIG. 66

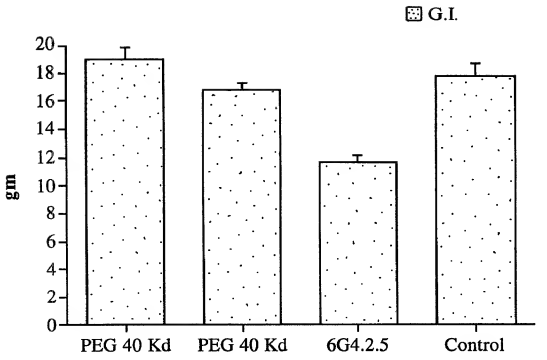

G.I.
gm
20
18
16
14
12
10
8
6
4
2
0
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 67

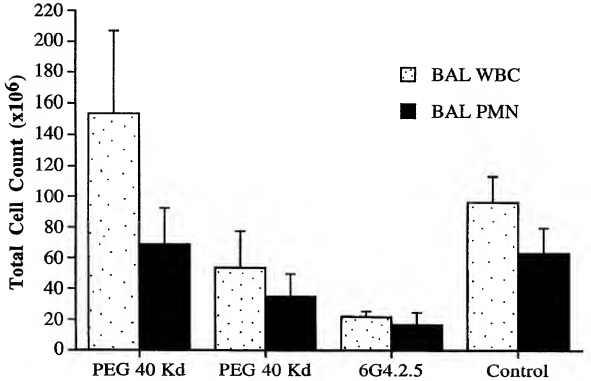

Total Cell Count (x10⁶)
220
200
180
160
140
120
100
80
60
40
20
0
BAL WBC
BAL PMN
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 68

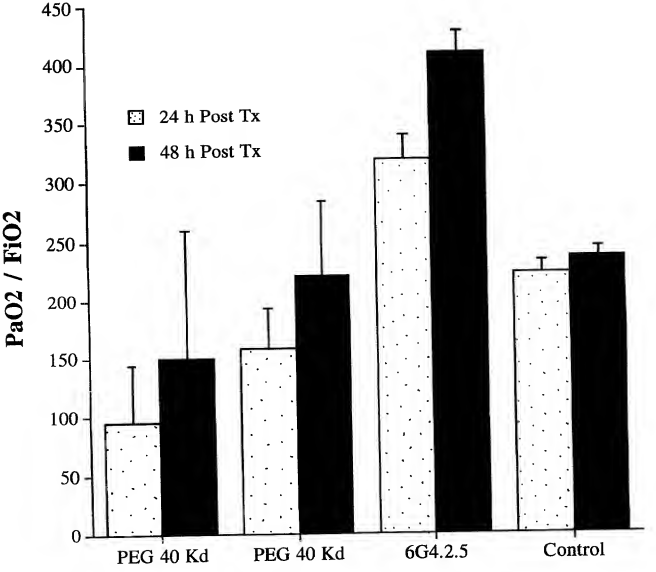

450
400
350
300
250
200
150
100
50
0
PaO2 / FiO2
24 h Post Tx
48 h Post Tx
PEG 40 Kd
PEG 40 Kd
6G4.2.5
Control

FIG. 69

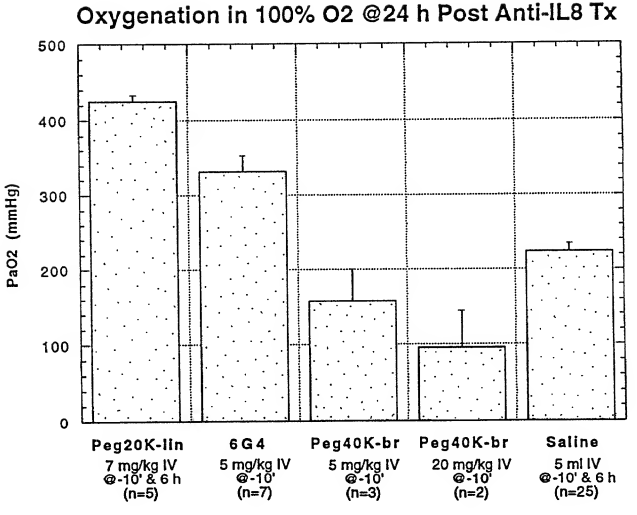

Oxygenation in 100% O2 @24 h Post Anti-IL8 Tx
PaO2 (mmHg)
500
400
300
200
100
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=2)
Saline
5 ml IV
@-10' & 6 h
(n=25)

FIG. 70A

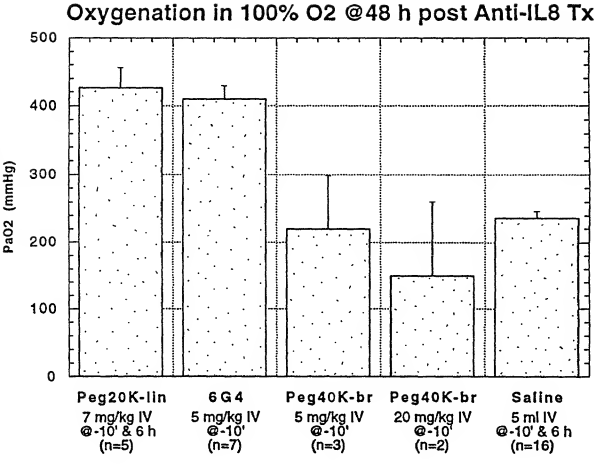

Oxygenation in 100% O2 @48 h post Anti-IL8 Tx
PaO2 (mmHg)
500
400
300
200
100
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=2)
Saline
5 ml IV
@-10' & 6 h
(n=16)

FIG. 70B

Sheet 1 of 1

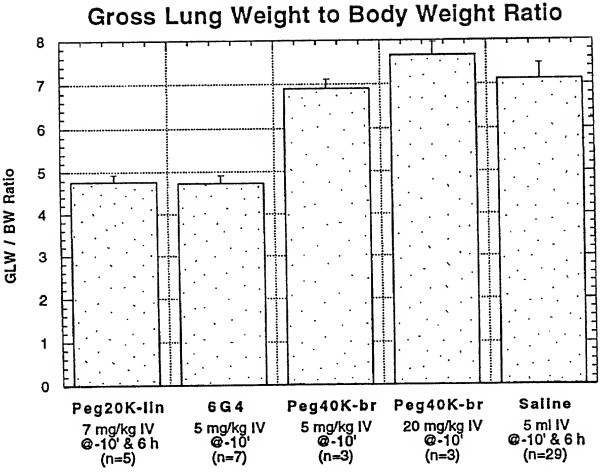


FIG. 70C

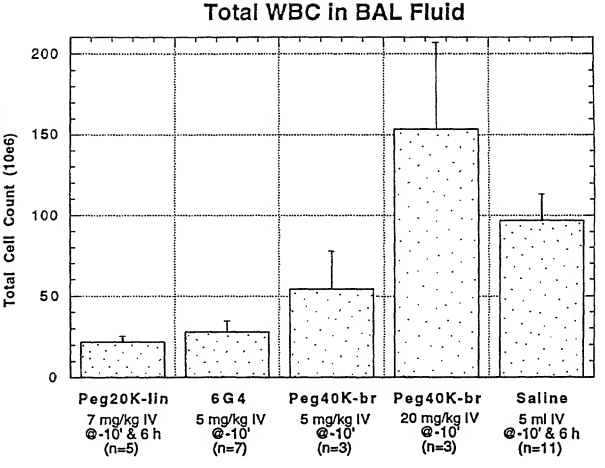

Total WBC in BAL Fluid
Total Cell Count (10e6)
200
150
100
50
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=3)
Saline
5 ml IV
@-10' & 6 h
(n=11)

FIG. 70D

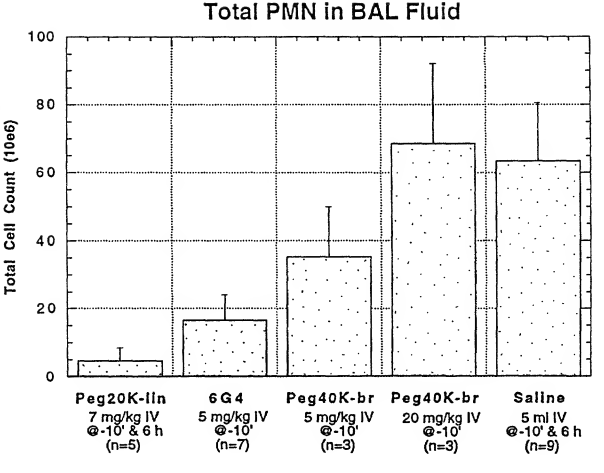
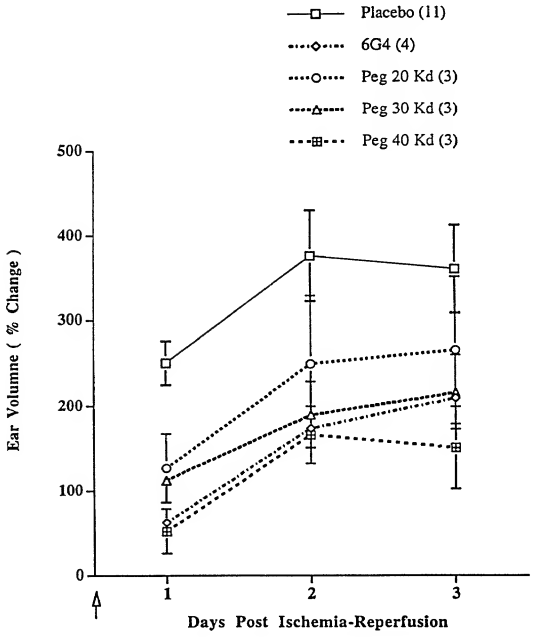

Total PMN in BAL Fluid
Total Cell Count (10e6)
100
80
60
40
20
0
Peg20K-lin
7 mg/kg IV
@-10' & 6 h
(n=5)
6G4
5 mg/kg IV
@-10'
(n=7)
Peg40K-br
5 mg/kg IV
@-10'
(n=3)
Peg40K-br
20 mg/kg IV
@-10'
(n=3)
Saline
5 ml IV
@-10' & 6 h
(n=9)

FIG. 70E

The Effect of Pegylated Anti-IL-8 in the Rabbit Ear model of Ischemia-Reperfusion Injury



Anti-IL-8 formulations : Single Dose (5 mg/kg) administered IV at time of reperfusion

FIG. 71